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THE JOURNAL OF INDUSTRIAL HYGIENE

AND ABSTRACT OF THE LITERATURE

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Occupational Diseases Reported to the Ohio State Department of Health for the Five-Year Period Ending June 30, 1925

The Estimation of Zinc in Biologic Material

The Normal Occurrence of Zinc in Biologic Materials: A Review of the Literature, and a Study of the Normal Distribution of Zinc in the Rat, Cat, and Man

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OCCUPATIONAL DISEASES REPORTED TO THE OHIO STATE DEPARTMENT OF HEALTH FOR THE FIVE-YEAR PERIOD ENDING JUNE 30, 1925*

EMERY R. HAYHURST, M.D., PH.D.

Professor of Hygiene, Ohio State University, and Consultant in Industrial Hygiene, Ohio State Department of Health

AND

DANIEL J. KINDEL, B.S., M.D.

Chief, Division of Industrial Hygiene, Ohio State Department of Health

INTRODUCTION

WE PRESENT herewith statistical summaries and analyses of occupational diseases reported to the State Director of Health of Ohio under the Occupational Disease Reporting Law during the five-year period ending June 30, 1925. The study covers both males and females and includes only those reported on the official Certificate of Industrial or Occupational Disease (see Figure 1), known as the "standard" blank, and used in several other states. Our twelve years' experience with the reporting form has impressed us with its worth. It has not seemed fit to change any item of inquiry on it during this period of time. The instructions for filling out the Certificate and the law requiring reporting of occupational diseases, which became effective in May, 1913, and was amended Feb. 4, 1920, are printed on the reverse side of the blank (see page 145).

* Received for publication Dec. 3, 1925. Accepted for presentation at the Annual Meeting of the Ohio Academy of Science, Medical Section, Columbus, Ohio, April 9 and 10, 1926.

WRITE PLAINLY WITH INK—THIS IS A PERMANENT RECORD

N. B.—Every item of information should be carefully supplied. The exact statement of OCCUPATION is very important. Physicians should state DIAGNOSIS in plain terms. See instructions on back of certificate.

OHIO DEPARTMENT OF HEALTH

CERTIFICATE OF INDUSTRIAL OR OCCUPATIONAL DISEASE

NAME OF PATIENT _____

ADDRESS: Street and No. _____ City or Village _____

PERSONAL AND STATISTICAL PARTICULARS

Sex	Age	Color	Country of Birth

Single, married, widowed or divorced (*write* the word)

Occupation

(a) Present trade, profession or work _____

Particular kind of work in such trade, etc. _____

Date of entering present occupation _____

Employer's name _____

Address _____

Business (kind of goods made or work done)

(b) Previous occupations:

Name of occupation	Entered (year)	Left (year)

Previous illnesses, if any, due to occupation:

Disease or illness	Year

MEDICAL CERTIFICATE OF DISEASE

Diagnosis of present illness _____

Chief symptoms and conditions _____

Date first symptoms appeared _____

Complicating Diseases (such as alcoholism, syphilis, tuberculosis, etc.) _____

Additional facts _____

Date of diagnosis _____, 192__

(Signed) _____, M.D.

_____, 192__ (Address) _____

Mail to STATE DIRECTOR OF HEALTH, Columbus.

For Instructions See Other Side

FIG. 1.—The standard reporting form for occupational diseases in Ohio (obverse side).

OHIO DEPARTMENT OF HEALTH

COLUMBUS

INSTRUCTIONS FOR FILLING OUT CERTIFICATE

Present Occupation. *Precise* statement of occupation is very important so that the relative healthfulness of various pursuits may be known. It is necessary to know both general trade or profession (for example, *printer*) and also the particular kind of work or branch of the trade (as *hand compositor* or *linotype operator*).

Date of entering present occupation is important to determine how long the worker may have been exposed to the hazard before contracting the disease.

Employer's name, address and business are necessary to ascertain distribution of occupational diseases by industries, many trades (e.g., machinists) being common to different industries.

Previous Occupations need to be known, if possible, because present illness may be due to a former rather than present occupation. Give simply the name of each distinct occupation which the patient may have followed, with the year he entered and the year he left.

Previous Illnesses. This refers either to previous attacks of present disease, or to any other disease, *due to occupation*. All that is required is the name of each such disease or illness with the year in which it occurred.

Medical Certificate. Only the last two items specified for this require any explanation. In making these reports it is necessary to consider the possible influence of factors other than occupation as causes of the disease. For this reason any *complicating diseases* should be noted, such, for example, as alcoholism or syphilis in connection with arteriosclerosis in cases of lead or other metal poisoning. The possible effect of other factors, such as poor hygienic conditions in the home, or other personal conditions, must be considered, and when discoverable should be noted under *additional facts*.

AN ACT—To Require the Reporting of Occupational Diseases—(As amended February 4, 1920)

Be it enacted by the General Assembly of the State of Ohio:

Report of occupational diseases by physicians

SECTION 1243-1. Every physician in this state attending on or called in to visit a patient whom he believes to be suffering from poisoning from lead, phosphorus, arsenic, brass, wood alcohol, mercury or their compounds, or from anthrax or from compressed air illness and such other occupational diseases and ailments as the state department of health shall require to be reported, shall within forty-eight hours from the time of first attending such patient send to the state commissioner of health a report stating:

When and to whom to be made

(a) Name, address and occupation of patient. (b) Name, address and business of employer.

(c) Nature of disease. (d) Such other information as may be reasonably required by the state department of health.

The reports herein required shall be made on, or in conformity with, the standard schedule blanks hereinafter provided for. The mailing of the report, within the time required, in a stamped envelope addressed to the office of the state commissioner of health, shall be a compliance with this section.

Blanks for report

SECTION 1243-2. The state department of health shall prepare and furnish, free of cost, to the physicians included in the preceding section, standard schedule blanks for the reports required under this act. The form and contents of such blanks shall be determined by the state department of health.

Such reports not evidence

SECTION 1243-3. Reports made under this act shall not be evidence of the facts therein stated in any action arising out of the disease therein reported.

Copy of report to be transmitted to proper official

SECTION 1243-4. It shall furthermore be the duty of the state department of health to transmit a copy of all such reports of occupational disease to the proper official having charge of factory inspection.

Penalty

SECTION 1243-5. Whoever being a physician practicing in the state of Ohio, neglects or refuses to make and transmit to the state commissioner of health any report provided for in section 1243-1 of the General Code shall be fined not to exceed one hundred dollars or imprisoned for not to exceed ninety days, or

both, but no person shall be imprisoned under this section for a first offense and the prosecution shall always be as and for a first offense unless the affidavit upon which the prosecution is instituted contains the allegation that the offense is a second or repeated offense.

NOTE—In addition to the diseases or disabilities provided for in Section 1243-1 of the above law, the regulations passed by the Public Health Council on February 27, 1920, provide in Regulation 2 for the reporting of "any disease or disability contracted as a result of the nature of the person's employment, including the following diseases or disabilities and not excluding others:

Anilin poisoning
Benzine (gasoline) poisoning
Benzol poisoning
Bisulphide of carbon poisoning
Carbon monoxide poisoning
Dinitrobenzene poisoning
Naptha poisoning
Natural gas poisoning
Turpentine poisoning

During the nineteen months ending Nov. 30, 1914, there were found by the special occupational disease investigators for the (then) State Board of Health a total of 864 occupational diseases of positive (specific, noncontroversial) character to which 211 of tentative character, 301 of industrial tuberculosis, and 39 miscellaneous were added, making a total of 1,415 cases (1). At that time lead poisoning was by far the chief affliction reported, totaling 544 of the 864 positive cases. Brass chills contributed 117 cases; dermatoses made up only 41 cases; and benzine and benzol poisoning, 33 cases.

From Dec. 1, 1914, to June 30, 1920, 873 additional cases of positive occupational diseases had been reported, bringing the total to 1,737 *bona fide* cases of occupational diseases, not including tentative cases and cases of reputed "industrial tuberculosis." The present study adds 3,226 more approved cases, making a total of 4,963 reported between May, 1913, and June 30, 1925—a period of twelve years and two months.

Reporting of Cases Following Enactment of the Compensation Law, Aug. 4, 1921

Following the enactment of the compensation law (2) which schedules fifteen definite occupational afflictions for compensation and which became effective Aug. 4, 1921, the reporting of occupational diseases to the State Director of Health took on a very material increase. The section containing this schedule reads as follows:

The following diseases shall be considered occupational diseases and compensable as such, when contracted by an employe in the course of his employment in which such employe was engaged at any time within twelve months previous to the date of his disablement and due to the nature of any process described herein.

SCHEDULE

Description of Disease or Injury	*Description of Process*
1. Anthrax	Handling of wool, hair bristles, hides and skins.
2. Glanders	Care of any equine animal suffering from glanders; handling carcass of such animal.
3. Lead poisoning	Any industrial process involving the use of lead or its preparations or compounds.

SCHEDULE (*Continued*)

Description of Disease or Injury	*Description of Process*
4. Mercury poisoning	Any industrial process involving the use of mercury or its preparations or compounds.
5. Phosphorus poisoning	Any industrial process involving the use of phosphorus or its preparations or compounds.
6. Arsenic poisoning	Any industrial process involving the use of arsenic or its preparations or compounds.
7. Poisoning by benzol or by nitro- and amido-derivatives of benzol (dinitrobenzol, anilin and others)	Any industrial process involving the use of benzol or a nitro- or amido-derivative of benzol or its preparations or compounds.
8. Poisoning by gasoline, benzine, naphtha, or other volatile petroleum products	Any industrial process involving the use of gasoline, benzine, naphtha, or other volatile petroleum products.
9. Poisoning by carbon bisulphide	Any industrial process involving the use of carbon bisulphide or its preparations or compounds.
10. Poisoning by wood alcohol	Any industrial process involving the use of wood alcohol or its preparations.
11. Infection or inflammation of the skin on contact surfaces due to oils, cutting compounds or lubricants, dust, liquids, fumes, gases or vapors	Any industrial process involving the handling or use of oils, cutting compounds or lubricants, or involving contact with dust, liquids, fumes, gases or vapors.
12. Epithelioma cancer or ulceration of the skin or of corneal surface of the eye due to carbon, pitch, tar or tarry compounds	Handling or industrial use of carbon, pitch or tarry compounds.
13. Compressed air illness	Any industrial process carried on in compressed air.
14. Carbon dioxide poisoning	Any process involving the evolution or resulting in the escape of carbon dioxide.
15. Brass or zinc poisoning	Any process involving the manufacture, founding or refining of brass or the melting or smelting of zinc.

In fact, this compensation feature, although negotiated entirely through another state department, that of industrial relations, influenced reporting very much more than did the penalty clause (2, Section 1243-5, General Code) which was enacted in 1919, but which proved practically no stimulus. This is shown from the fact that for the period Aug. 4, 1921, to June 30, 1925—four years, lacking thirty-four days—the State Director of Health received 2,689 reports of approved cases as compared to 2,274 reports received prior to that time (or from May, 1913)—a period of eight years, plus about ninety days. Variations in industrial activity could not have accounted for the difference to any perceptible degree.

Since the inauguration of the compensation law necessitated double reporting of the fifteen occupational diseases scheduled—*i.e.*, to the State Director of Health and to the State Department of Industrial Relations—it was foreseen that many cases would be reported to one agency and

not to the other. An attempt was made at the time to offset this difficulty by the preparation of duplicate reports, one to be sent by the reporting physician to each agency, but this did not work out effectively. Likewise, owing to shortage of personnel in both state departments, it was not possible to transcribe or to compare reports received by each. We can, therefore, give totals only at this writing. During the period quoted (*i.e.*, Aug. 4, 1921, to June 30, 1925), whereas the Director of Health received 2,689 cases, the Department of Industrial Relations received 2,841 cases reported for compensation. We are unable to say what percentage of the cases included in either figures is duplicated in the other. Roughly, however, over half of the cases reported for compensation have not been reported to the Director of Health and therefore constitute some 1,400 additional cases of occupational diseases which should be added to the figures presented in this study if the total reported cases is wanted; in other words,

$$4{,}963 + 1{,}400 \pm = 6{,}363\pm$$

or the approximate total number of cases of specific occupational diseases reported in Ohio by physicians on official blanks during the approximately twelve-year period ending June 30, 1925.

Studies Made Prior to July 1, 1924

A rather elaborate tabulation of the occupational diseases reported in the state survey of 1913 to 1914 is given in the report mentioned (1, pp. 361–377) in which the cases are classified by industry, trade process, disease or disability reported, and the number of positive and tentative cases as well as the probable percentage total of those exposed in each trade process. Nothing so complete as this can be included in the present study, principally because so many different persons have reported on trade processes (in place of the special investigators during the survey) and because we have relatively no idea of the average number employed in the various industries and trade processes during the five-year period which the present paper encompasses. The figures are presented, therefore, simply as absolute values from which little respecting case rate can be derived.

One of us (3) reported on the status of occupational diseases in Ohio bringing the total cases reported to both state agencies up to the date of Dec. 31, 1923, and discussing particularly the retrospect and prospect of the situation, laying stress on the necessity of the inclusion of some twenty afflictions in the compensation schedule, for apparently greater equity in that matter.

Changes Effected by the Compensation Ruling of July 1, 1925

In the Rules and Rates of July 1, 1924 (4), the 774 classifications were grouped into seventeen schedules for occupational disease as follows:

Schedule	*Premium Rate per $1,000 Payroll*
Food and beverages	$ .01
Chemical	.30
Wood and metal products	.60
Coal, ores, and stone	.01

Schedule	*Premium Rate per $1,000 Payroll*
Construction	.01
Transportation and utilities	.01
Rubber	1.00
Leather	.20
Wood	.20
Oils	.05
Metal	.20
Textiles	.01
Paper	.30
Pottery and glass	.20
Stores	.01
Lead	4.00
Miscellaneous	.02

Obviously, the effect of such grouping was to stimulate interest in reducing occupational diseases where the rates were high.

Because of an actuarial difficulty, however, in setting aside fractions of a cent to the reserve fund for occupational diseases in the case of some groups (representing hundreds of classifications) paying as little as 1 cent per $1,000 of payroll, an entire change in the method of premium rating for occupational diseases was made by the legislature of 1925 (Eighty-Sixth General Assembly).

According to the Rules and Rates effective, therefore, July 1, 1925 (5), the occupational disease rating method becomes a flat rate of $1\frac{1}{2}$ cents per $100 of payroll for all classifications. There are 750 active industrial classifications which cover all industrial and trade processes in the state. Furthermore, while "merit rating" applies to accident exposures—*i.e.*, the fixing of annual premiums for individual employers depends on the past year's experience of such employers—under the new legislation, "merit rating" does not apply to occupational disease experience. The result of these innovations is that all employers pay the same premium for occupational diseases, *viz.*, $1\frac{1}{2}$ cents per $100 of payroll, irrespective, for example, of whether they are storage battery manufacturers or simply employers of mercantile help, and no credit or penalty is fixed in premium rates with regard to occupational disease experience.

Division of Safety and Hygiene.—The same legislature created a new division which has been termed the Division of Safety and Hygiene and which has been placed directly under the State Industrial Commission, a separate body from the Department of Industrial Relations. A part of the duties of this new division is to bring about a reduction in occupational diseases through preventive measures. Thus the state offers services similar to those of private insurance companies in similar fields. To finance the new division, 1 per cent. of total premiums paid into the state fund each year is set aside. This amounts to something over $100,000 a year.

It is impossible to foresee at the present time what effects the new system of a flat rate premium for occupational diseases and the efforts of the new Division of Safety and Hygiene will have on several important features such as (1) the reporting of occupational diseases; (2) the true prevalence of occupational diseases; (3) the tendency to change borderline cases of accidents to occupational disease because of the much lower premium rate (boils and skin infections, for examples, which have up to the present time often

been classed as accidents); (4) the tendency to increase the occupational disease schedule of fifteen afflictions by the addition of new diseases for compensation purposes; and (5) the prevention of occupational diseases. It will undoubtedly take several years to see the trend in these matters. In the meantime the experience of the first eight months indicates an increase in the reporting of occupational diseases, but there are other factors which have entered into this; also it is not probable that the knowledge of the new classification and rating method has become widespread enough as yet to produce its effects. At all odds there is no question but that the actuarial difficulties have been greatly simplified and that an adequate fund with proper reserve is being accumulated for the compensation of occupational diseases.

In the meantime the State Director of Health continues to receive reports of all occupational diseases of whatsoever character and source and to transmit copies of these reports to the chief of the Division of Factory Inspection (a division under the Department of Industrial Relations). The Division of Industrial Hygiene in the State Department of Health also continues to make a study of all diseases which might of necessity come to be classified as occupational diseases.

Statistics of Occupational Diseases Reported during the Five-Year Period Ending June 30, 1925

It is convenient to divide the occupational diseases reported in Ohio into two groups: those falling under the schedule for compensation as administered by the State Department of Industrial Relations (2) which became operative Aug. 4, 1921; and those not in the schedule and therefore noncompensable.

It is to be noted that in the present analysis we have gone back a little over a year prior to the application of the Compensation Act (*i.e.*, to July 1, 1920); hence Table 1 shows such afflictions as might have been compensable had the present schedule for compensation been effective throughout the five-year period ending June 30, 1925. In other words, this is a report on the prevalence and kind of occupational diseases as officially reported rather than on the compensation of occupational diseases.

In order to clarify Table 1, and to establish the occupational relationship for the cases reported in so far as information is given in the original reports, the following explanation of the items is pertinent.

Compensable Occupational Diseases:

1. The case of anthrax in a male occurred in an inspector of hair and bristles; that in the female was reported in a handler of paint brushes in a blue print works.

3. Lead poisoning, see Table 2 and explanatory notes.

4. The case of mercury poisoning, male, occurred in a foreman in a thermometer works. The three cases of mercury poisoning in females all occurred in a thermometer works (two in "blowers" and one in a worker engaged in the process of filling bulbs with mercury).

6. The seven cases of arsenic poisoning (all males) were in workers engaged in making and handling tree

and plant sprays consisting mainly of lead arsenate. (See also lead poisoning in this process as given in Table 2, item 1.)

7. Poisonings in males by benzol or by nitro and amido derivatives of benzol (dinitrobenzol and others) were distributed as follows:

a. The two cases of acetanilide poisoning occurred in chemical and dye manufacturing.

b. The thirty cases of aniline poisoning were distributed as follows: twenty-one in chemical and dye manufacturing, eight in the rubber industry, and one not specified.

c. The seven cases of benzol poisoning occurred as follows: five in the rubber industry, one in chemical and dye manufacturing, and one in the manufacture of insulating materials.

d, e, f. The eleven cases of para-nitraniline, four of para-toluidine (also the one case in a female), and one of xylidine poisoning all occurred in a chemical works.

The twenty-two cases of benzol poisoning in females were all reported by the late Dr. E. B. Starr and occurred in a wholesale millinery manufacturing company (6).

8. The four reported cases of poisoning under gasoline, benzine, naphtha, or other volatile petroleum products (all males) were distributed as follows: one, by gasoline, in a dry cleaner; two, by benzine, in rubber workers; and one, by benzine, in a printing press feeder.

10. Three cases of wood alcohol poisoning (all males) were reported by a hospital receiving physician as, apparently, of occupational origin but other information was lacking.

11. Dermatitis in rubber workers is further subclassified for males and females and is discussed in connection with Tables 3 and 4, respectively.

12. The case of epithelioma of the lip occurred in a male carbon worker, employed by a carbon manufacturing company.

15. There were 14 cases of brass and zinc poisoning (all males) showing typical symptoms of zinc fume poisoning, such as fever, chills, sweats, etc. Of the 14 cases, 4 were diagnosed as brass poisoning, and occurred, 1 in a brass furnace tender, 1 in a brass molder, and 2 in brass welders. The 10 cases of zinc poisoning were distributed as follows: 7 in welders of galvanized steel drums, 1 in an acetylene cutter of zinc metal, and 2 in electricians working in a brass foundry. These last 2 cases should undoubtedly be classified under brass poisoning with the first 4 mentioned. They serve to show the ease with which brass (or zinc) fumes may produce their characteristic syndrome.

Noncompensable Occupational Diseases:

16. The case of actinomycosis reported was in a cobbler. Definite association with his occupation was not established.

17. The poisoning from ammonia gas occurred in a plumber who was cleaning ammonia pipes and might properly be classed as an accident.

18. One case each of occupational bronchitis in males was reported as due to: fumes from a plating solution; treating steel with heat; chlorine fumes from a swimming pool (in a custodian); fumes (probably sulphurous) from a mine dump; and one not specified. The case in the female

TABLE 1.—SUMMARY OF OCCUPATIONAL DISEASES BY SEX AND CAUSE, AS REPORTED TO THE OHIO STATE DEPARTMENT OF HEALTH[1] DURING THE FIVE-YEAR PERIOD ENDING JUNE 30, 1925

ITEM	CAUSE OF DISEASE	NUMBER OF CASES			
		Male		Female	
	Compensable[2]				
1	Anthrax		1		1
2	Glanders		...		...
3	Lead poisoning (see Table 2)		431		12
4	Mercury poisoning		1		3
5	Phosphorus poisoning		...		...
6	Arsenic poisoning		7		...
7	Poisoning by benzol or by nitro and amido derivatives of benzol (dinitrobenzol, aniline, and others):				
	a. Acetanilide	2		...	
	b. Aniline	30		...	
	c. Benzol	7	55	22	23
	d. Para-nitraniline	11		...	
	e. Para-toluidine	4		1	
	f. Xylidine	1		...	
8	Poisoning by gasoline, benzine, naphtha, or other volatile petroleum products:				
	a. Benzine	3	4	...	...
	b. Gasoline	1		...	
9	Carbon bisulphide poisoning		...		...
10	Wood alcohol poisoning		3		...
11	Infection or inflammation of the skin on contact surfaces due to oils, cutting compounds or lubricants, dust, liquids, fumes, gases or vapors:				
	a. Acids (rim truckers in rubber works)	2		...	
	b. Arsenic (chemical manufacture)	2		...	
	c. Brass (grinders)	2		...	
	d. Chromium salts (carbon paper)	...		1	
	e. Cyanide solution (platers)	4		...	
	f. Developer (photographers)	2		...	
	g. Dinitrobenzene (laborers in chemical works)	2	2,264	...	371
	h. Gasoline (garage hand)	1		...	
	i. Matches, manufacture	8		...	
	j. Oil dermatitis (machinists)	2		...	
	k. Oil furunculosis (machinists)	3		...	
	l. Plating solution (platers)	7		...	
	m. Rubber (see Tables 3 and 4)	2,227		370	
	n. Sand blasting (iron castings)	1		...	
	o. Steel drums (handling same)	1		...	

[1] Under Section 1243–1, General Code.

[2] Under the present law (Section 1465–68a).

TABLE 1.—*Continued*

ITEM	CAUSE OF DISEASE	NUMBER OF CASES	
		Male	Female
	Compensable—Continued		
12	Epithelioma—cancer or ulceration of the skin of the corneal surface of the eye due to carbon, pitch, tar or tarry compounds:		
	Epithelioma of lip (carbon worker)	1	...
13	Compressed air illness	...	...
14	Carbon dioxide poisoning	...	...
15	Brass and zinc poisoning:		
	a. Brass fumes	4	...
	b. Zinc fumes	10	...
		14	
	Total compensable	2,781	410
	Noncompensable[2]		
16	Actinomycosis	1	...
17	Ammonia gas poisoning	1	...
18	Bronchitis	5	1
19	Carbon monoxide poisoning	7	...
20	Dye poisoning	1	...
21	Hydrocyanic acid poisoning	5	...
22	Laryngitis (acid dipper)	1	...
23	Manganese poisoning	1	...
24	Metal poisoning, chronic	1	...
25	Necrosis, end of finger (acid dipper)	1	...
26	Nicotine poisoning	...	1
27	Occupational neuroses	3	...
28	Phenol poisoning	1	...
29	Phthisis	1	...
30	Pneumonokoniosis	1	...
31	Ulcerated throat	1	...
32	Undefined	1	...
33	Varnish fumes	1	...
	Total noncompensable	33	2
	Total compensable	2,781	410
	Total noncompensable	33	2
	Totals	2,814	412
	Grand total, both sexes	3,226	

was laid to dust from straw in a basket factory.

19. The seven cases of carbon monoxide poisoning (all males) were evidently not accidents—*i.e.*, eventualities of a single time and place occurrence—and were distributed as follows: laborer working in a sewer;

helper on electric motors; stationary engineer; heater man (regulating gas ovens in a rubber company); bricklayer working in a furnace; blacksmith (sleeping over gas ovens); boiler tender (in refining company). The question of chronic disease due to carbon monoxide poisoning is always a disputable one.

20. The case of dye poisoning was not further specified.

21. Mixing and handling feed and grain were responsible for the five cases of hydrocyanic acid poisoning, the substance undoubtedly being previously used as a disinfectant. The cases were nonfatal.

22. The case of laryngitis was reported in an acid (nitric) dipper; the industry was not specified. The case of necrosis of the end of the finger also occurred in an acid dipper (item 25).

23. The case of manganese poisoning occurred in a steel worker handling manganese.

24. The case of chronic metal poisoning occurred in a master mechanic engaged in brass and bronze manufacturing. This was probably an instance of mixed poisoning and could not be specified further.

26. The case of nicotine poisoning in a female was laid to the accidental splashing of nicotine on the lips while spraying nicotine mixture in a garden; as such, it would be better classified as an accident.

27. Two cases of occupational neuroses (diagnosed as myasthenia) were reported in shoe cutters and one in a railroad engineer.

28. A porter for a chemical company was reported as poisoned by phenol.

29 and 30. The case of phthisis occurred in a file grinder, and the case of pneumonokoniosis in a sweeper employed for ten years by a rubber company.

31. The case of ulcerated throat was reported in a janitor, and alleged as due to using a cleaning fluid.

33. Varnish fumes poisoned an automobile sander and painter.

Of the total of 2,814 cases of occupational diseases reported in males during the five-year period ending June 30, 1925, 2,781 came under the list of occupational diseases which are compensable under the law, leaving 33 not so included (see Table 1). Similarly, of the 412 reported for females during the same period, 410 were compensable and only 2 were not. Of the compensable diseases in the schedule (2) there were not reported for either sex any cases of *glanders, phosphorus poisoning, carbon bisulphide poisoning, carbon dioxide poisoning,* or *caisson disease.* We cannot state that these did not occur, but our general knowledge of the industries in the state would indicate that these afflictions were very rare, while glanders and caisson disease probably did not occur at all. As for the remainder, our records are by no means representative of the number of cases which have existed during these five years, many more than were reported doubtlessly having occurred.

Cases of Lead Poisoning

Table 2 gives the industries and trade processes associated with the reported cases of lead poisoning. The age distribution for males is shown in Figure 2. The preponderance of

TABLE 2.—LEAD POISONING BY INDUSTRIES, TRADE PROCESSES, AND SEXES, AS REPORTED TO THE OHIO STATE DEPARTMENT OF HEALTH[1] DURING THE FIVE-YEAR PERIOD ENDING JUNE 30, 1925

ITEM	SOURCE OF POISONING	NUMBER OF CASES			
		Male		Female	
1	Agricultural products:				
	Making and handling tree and plant sprays		10		...
2	Automobiles and parts:				
	Sanding	105			
	Polishing	1			
	Painting	5	114		...
	Spray painting	2			
	Scrollwork	1			
3	Brass and bronze products:				
	Brass molders	3			
	Grinders	2	7		...
	Machinists	1			
	Furnace tender	1			
4	Chemical manufacture:				
	Packing oxide	2			
	Grinding mill	1	4		...
	Mixing	1			
5	Electroplating and polishing on safes		1		...
6	Enameling and japanning:				
	Preparing	1			
	Enameling and making enamel	1			
	Enameling (brushing)	2	5	1	9
	Spraying	1		8	
7	Gasoline and oil:				
	Blending tetraethyl fluid		24		...
8	Glass:				
	Spraying	4	8		...
	Batch mixing	4			
9	Iron and steel:				
	Spraying iron structural work	1	2		...
	Foreman	1			
10	Metal, solder, and alloys:				
	Refining metal	9			
	Installing machinery	1	12		...
	Night watchman	1			
	Handling lead	1			
11	Paint and varnish manufacture:				
	Filler of paint cans	1			
	Trucking	1	3		...
	Mixer	1			
12	Painters:				
	Mixer	2	3		...
	Painting dairy machinery	1			

[1] Under Section 1243-1, General Code.

TABLE 2.—*Continued*

ITEM	SOURCE OF POISONING	NUMBER OF CASES		
		Male		Female
12	Painters—*Continued*			
	Painting butchers' machinery	1		
	Self-employed	1		
	House painters	5		
	Repairing barrels	1	17	...
	Interior decorators	4		
	General	3		
	Handling castings	1		
	Flat work	1		
13	Pottery and terra cotta:			
	Dipping		1	3
14	Printing and publishing:			
	Type reclaimer	1		
	Hand typesetting	1		
	Make-up foreman	1	5	...
	Monotype operator	1		
	Stereotyper	1		
15	Rubber goods:			
	Compounders	8		
	Hard rubber press operator	1	11	...
	Not specified	2		
16	Smelting and refining:			
	Refining lead		4	...
17	Storage batteries:			
	Assemblers	15		
	Burners	13		
	Pasters	26		
	Rackers	23		
	Laborers	11		
	Forming	21		
	Smelting	2		
	Checkers	2		
	Drying room	1		
	Mixers	13		
	Clerk	1	145	...
	Cleaner	1		
	Sorter	4		
	Molders	2		
	Separating	1		
	Acid	1		
	Janitors	2		
	Scrapers	1		
	Sawing	2		
	Punch press	1		
	Not specified	2		
18	Tin can manufacture:			
	Solder bath process		1	...

TABLE 2.—*Concluded*

ITEM	SOURCE OF POISONING	NUMBER OF CASES			
		Male		Female	
19	White lead manufacture:				
	Take-out gang	17			
	Picking up lead	2			
	Kiln	3			
	Yard work	1			
	Oxide department	13			
	Repair gang	2			
	Water stones	3	54	.....	
	Discharging	3			
	Building	1			
	Pulling lead out of buckets	1			
	Packing	1			
	Can room	3			
	Not specified	4			
Totals			431		12

cases in storage battery manufacturing, 145, and in automobile manufacturing, 114, is at once apparent, while the 54 cases in the manufacture of white lead, and 24 in the blending of tetraethyl lead for gasoline, the 20 and 5 in painters and enamelers, respectively, in males, the 23 in nonferruginous metal workers (7 in brass and 16 otherwise, items 10 and 16), and the 11 in rubber workers are all prominent. Of the 9 cases in female enamelers, 1 occurred in brushing and 8 in spraying.

Attention should be called, also, to the danger of lead poisoning in the making and handling of tree and plant sprays, 10 cases, where the total number employed is relatively small compared, for instance, with the large number employed in the printing and publishing industry in the state, which yielded only 5 cases only 1 of which occurred in a typesetter. The significance of sprays and spraying is shown for males also in the glass industry, 4 cases, and in the iron and steel industry, 1 case, in addition to cases among those regularly employed in painting, decorating, and enameling. On the other hand the small number of cases reported in chemical manufacture, 4, and in paint and varnish manufacture, 3, undoubtedly shows good control in these industries.

The small number of cases of lead poisoning reported in general (or house) painters and decorators, not over fourteen all told, an average of less than three a year, is remarkable in a state where perhaps 25,000 such workers are to be found. Undoubtedly acute cases of plumbism seldom occur, while chronic cases are not recognized. Also, since such workers are "independents," and therefore many are not under the compensation law, they seek their private physicians who overlook the reporting of their plumbism diagnoses. The sum and

substance, however, is that general painters have little in the way of official statistics to back up any claims concerning an occupation which is hazardous to health—a matter which painters' organizations should look

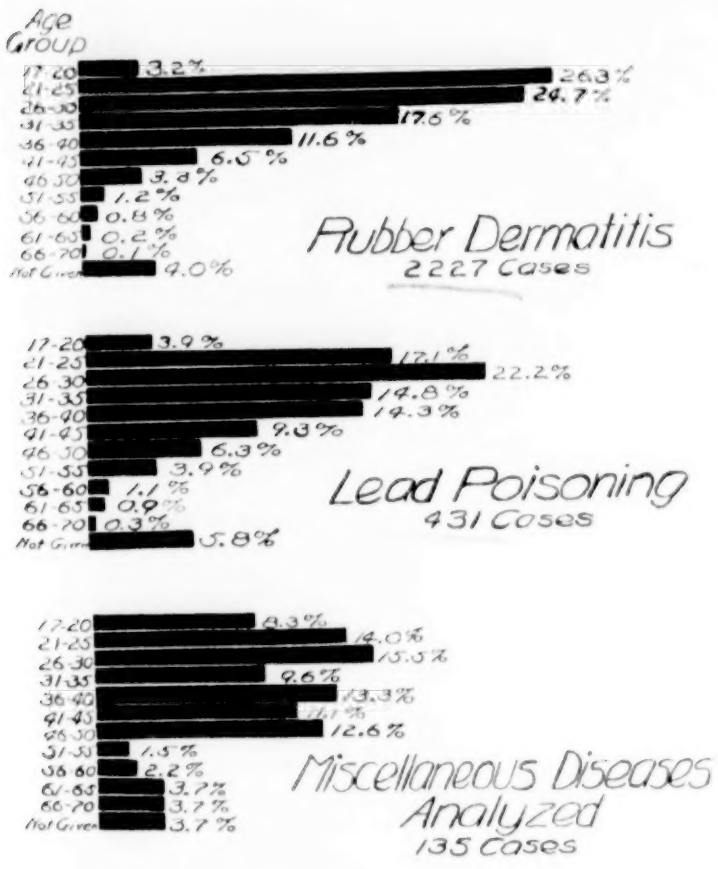

FIG. 2.—Reported cases of occupational diseases among males in Ohio during the period July 1, 1920, to June 30, 1925, by age groups.

into so that they may instruct their members on the reporting of occupational diseases if such really exist.

Our four cases, one male and three female, of lead poisoning in the pottery and terra cotta industry most certainly mean lack of reporting, although acute or outstanding cases are undoubtedly rare in this industry as at present conducted in the state. On the other hand, chronic poisoning was prominent in the state survey of occupational diseases made by the State Board of Health in 1913 to 1914 where Albaugh (reported by Starr (7)) tabulates 109 cases as found by the investigators among 2,585 examined, or a case rate of 4.2, and especially in the survey made by the United States Public Health Service in 1919 (8) where 139 positive cases were found among 1,809 examined, or a case rate of 7.7 per cent.

The 431 cases of lead poisoning were reported by sixty-seven companies. Of these, one company reported 104 cases (all of which occurred within a few months in an automobile body manufacturing plant, on the initiation of a sandpapering process without due precautions, promptly corrected with excellent results); two companies reported 60 cases each, one 39, one 24, one 15, one 12, one 11, and the remaining fifty-nine companies reported from 1 to 7 cases each.

There has been a marked falling off in cases of lead poisoning in several large companies which have come under the provisions of the "lead law" (2, Section 6330–1 to 6330–12), also known as the "standard bill for the prevention of occupational diseases with special reference to lead poisoning," prepared by the American Association for Labor Legislation, a law which has been operative in Ohio since the spring of 1913.

Dermatitis among Rubber Workers

Table 3 shows the various rubber processes engaged in by males in which dermatoses occurred (for age distribution see Figure 2). Owing to the various names given the same process in different rubber companies, some trade processes are undoubtedly listed under two or more different headings in this table, but the absolute numbers are not duplicated. The average number of both sexes em-

TABLE 3.—DERMATOSES AMONG MALE RUBBER WORKERS BY TRADE PROCESSES, AS REPORTED TO THE OHIO STATE DEPARTMENT OF HEALTH[1] DURING THE FIVE-YEAR PERIOD ENDING JUNE 30, 1925

Five or more cases, as indicated, in each of the following:	
Assembling stock	32
Bias machine operatives	26
Builders and helpers (tires)	293
Calender men	155
Checkers	14
Cleaning molds	12
Curing	112
Cutters	45
Drying rubber	10
Elevator operators	7
Finishers	190
Foremen	8
Grinding and mixing	129
Handling tires	5
Hose makers	13
Inspectors	33
Machinists	12
Bead makers	22
Mill men	116
Mold men	56
Not specified (principally 1920, 1921)	277
Oilers	7
Painters	8
Porters	16
Packers	5
Preparing stock	11
Reclaiming department	6
Repairing stock	15
Rimmers and helpers	42
Shoemakers	18
Soap stoners	12
Refuse sorters	7
Splicers	31
Spreader-wind-ups	8
Squad[2]	14
Stockroom men	16
Strippers	49
Truckers	101
Tube cementers	29
Tube makers, trimmers, valvers	21
Tube testers	5
	1,988

TABLE 3.—*Continued*

Total forwarded	1,988
Turners	5
Turning spinning bowls	5
Utility men	11
Warm-up men	60
Washers and varnishers	21
Wrappers	14
	2,104
Four each in the following: belt makers, connections men, hand buffers, jar builders, liner cleaners, refiners, shipping men, steam men	32
Three each in the following: bolters, clerks, gum gagers, pool workers, press operators, instrument repair men	18
Two each in the following: crane operators, electricians, gum storeroom men, crude rubber handlers, hoist men, laboratory workers, tire layers, pipe fitters, repair men, riggers, stitch machine operators, tread machine operators, truck drivers	26
One each in the following: air bag valve stem operator, blue printer, book repair man, boring mill, bottle maker, bead buffer, buff motors, bag builder (air), battery cell builder (hard rubber), car bracer, casting tin, conveyor man, cracker, deflator, development department, doper, drill pressman, drum changer, duster, engineer, fireman, hanger, heel tumbler, hydraulic operator, insulation operator, golf ball core maker, massing, mandrel tester, pigment selector, power table worker, rag roller, recorder, skiving (tubes), slab mill, slitting machine, steel worker, substation operator, switchboard operator, tire accessory maker, tire breaker-out, tire brusher, tractor driver, tread booker, treader, vacuum man, weigher, window washer	47
Total	2,227

[1] Under Section 1243–1, General Code.
[2] Not otherwise specified.

ployed in the rubber industry in the state during this five-year period may be set roughly at 75,000. The cause for dermatitis in rubber workers is assigned almost exclusively to the use of hexamethylenetetramine (urotropine), added as an organic accelerator for vulcanization.

TABLE 4.—DERMATOSES AMONG FEMALE RUBBER WORKERS BY TRADE PROCESSES, AS REPORTED TO THE OHIO STATE DEPARTMENT OF HEALTH[1] DURING THE FIVE-YEAR PERIOD ENDING JUNE 30, 1925

Assembling (tires): fabric 35, valve patches 3	38
Bias machine operatives	9
Building (tires): air bag valves 1, bands 3, beads 35	39
Bushings, making of	1
Cleaning liners	1
Clerks	2
Final finishing	20
Heel punchers, feeding	2
Inspecting fan belts, tubes, and heels	21
Labeling boxes	3
Packing:[2] accessories 1, bulbs 3, heels 6, rubber bands 1	11
Polishing hard rubber	1
Rubber, hard, repairing	3
Rubber workers[2]	16
Shoemaking	4
Stock cutting	3
Stock preparing	4
Scrub women	9
Treads (for tires): assemblers 51, cutters 31, splicers 49	131
Tubes (for tires): cementing 21, deflating 5, packing 12, polishing 2, splicing 3	43
Trimming	2
Washing and varnishing	6
Weighing rings	1
Total	370

[1] Under Section 1243-1, General Code.
[2] Not otherwise specified.

All the cases of rubber dermatitis in males were reported by fifteen companies. Of these, one company reported 1,460 cases, one 685, one 22, one 21, one 19, and the remaining ten companies reported from 1 to 8 cases each.

Table 4 is analogous to Table 3, giving the trade processes and occupations of females in the rubber industry. As in Table 3, varied names are given the same process in different rubber companies but the absolute numbers are not duplicated. It is notable that practically all of the processes engaged in by females differ from those of the males. In certain groups, as the table points out, cases of dermatitis among females are more numerous but the numbers so engaged are unknown.

All the cases of rubber dermatitis in females were reported by six companies. Of these, one company reported 262 cases, one 91, and the remaining four companies reported from 1 to 8 cases each. The two companies reporting the most dermatitis in females were the same as those reporting the most in males (see foregoing paragraph).

Results of Efforts to Secure Complete Reporting

Beginning with July 1, 1925, concerted efforts have been made by the State Director of Health to secure complete reporting. This is being effected, among other methods, by checking up on reports made to the Department of Industrial Relations and notifying the certifying physician (at the same time supplying him with reporting blanks) of the requirements under the penalty clause

(2, Section 1243). The results so far have been almost perfect without having to enforce the penalty for neglecting to report. While rubber dermatoses still continue to lead, a great increase is appearing in types of cases. Moreover, reports are coming in from industries and establishments which have never before reported occupational diseases, and from many more physicians, some from unexpected quarters.

It seems that in spite of the publicity given to occupational diseases in the state for over twelve years and in spite of the circularization of physicians, who are the essential reporting agents, with literature and report blanks, the situation is still practically one of ignorance of the law on the part of nearly all of the physicians concerned. This is shown by the fact that physicians frequently send in a number of reports of cases in addition to the one (or so) particularly called to their attention by the Director of Health, in the present checking back.

It should be pointed out, further, that a study of Table 1 shows either that the vast majority of occupational diseases reported to the State Director of Health are compensable or that physicians have been reporting only those occupational diseases which are compensable and omitting others; *i.e.*, physicians are rarely observing the clause which occurs in Regulation 2 of the Ohio Sanitary Code (9) (in *italics*):

> Regulation 2. *Diseases and disabilities to be reported.* The diseases and disabilities herein named and classified are declared dangerous to the public health, are made notifiable, and the occurrence of cases or suspected cases in Ohio shall be reported as provided in the following regulations.
>
> Class "F"
>
> *Any disease, disability or ailment contracted as a result of the nature of the person's employment*, including the following diseases, disabilities or ailments *and not excluding others:*

The fact that occupational diseases which are not compensable do not tend to be reported is unfortunate, as it is exactly from the experiences to be accumulated from these cases that most of the evidence must be obtained for extending the present schedule wisely.

Commercial Significance of Health Control

Compensation for occupational diseases is a principle generally acknowledged as correct. Such afflictions, however, constitute a very small part of the sickness disability which occurs among workers. Recently Sappington (10) found that sickness causes twenty times as many cases of absenteeism as do accidents and is responsible for seven times as much loss of time from work.[1] True, the greater portion of sickness disability among workers is extra-industrial in nature and is equally as prevalent among adults of like ages and sexes in the community, but a large part is measurably influenced by industrial environment and methods of personnel supervision and, as such, is capable of

[1] This occurred in a typical industrial plant in Boston employing an average of 2,233 workers during the period 1918 to 1922, showing an average annual labor turnover of 20 per cent., the proportion of females to males being about 1 to 4.5, and about 90 per cent. of the employees being American born. Rejections had also previously been made, by physical examinations at the time of hiring, of those having serious defects.

considerable reduction in the matter of days of absence from work.

Dublin (11) gives conclusions based on incidence of illness among policyholders of the Metropolitan Life Insurance Company of New York. The average loss of working time was eight days per annum for gainfully employed men. Inasmuch as a great deal of illness goes unrecorded, the actual loss was probably much heavier. Furthermore, men who are engaged in industry and whose daily wages are vital to their families will often continue to work under conditions of personal health which would very properly impel clerical and professional employees to stop work and seek the advice of a medical man. Among industrial workers the death rate from accidental causes is about two and one-half times that for the nonindustrial group. Tuberculosis is more important than accidents, and is responsible for a loss of between eighteen months and two years in the longevity of workers. Industrial employment is probably the most important single factor in the tuberculosis death rate. Pneumonia is twice as high among industrial as among nonindustrial workers and is therefore considered an added industrial hazard. Further research is necessary, however, to show the relation between the development of pneumonia and industry. In a similar manner other conditions such as cerebral hemorrhage, Bright's disease, and organic heart disease show strikingly the effects of industrial exposure. The death rates are two and three times as high as in the nonindustrial groups during the active working years of life.

Some companies in Ohio, after commensurate organization, have cut the average annual sickness absence from the customary seven or eight days per person per year to two-thirds of that figure and even less, and we note that one company[2] in its annual report for 1924 claims a reduction to as low as 23.5 hours per employee for the year 1924, with a remark that this is an increase of only 0.2 per cent. over the year 1923.

As an example in environmental control, the New York State Commission on Ventilation (12) reports that a difference in room ventilation methods effecting a change of only 2°F. in temperature was found to make a difference of 18 per cent. in absence due to respiratory diseases in schoolchildren, and a difference of 70 per cent. in total illness from respiratory diseases. Truly, we are in an era when the fundamental importance of certain apparently trifling things is being recognized in many vicissitudes of life and business.

Collis (13) estimates that industrial medicine properly applied could effect a saving each year on labor turnover in England of from sixty to seventy million pounds; on lost time, of fifty to sixty million pounds; and through industrial convalescence of many millions more. He suggests that £140,000,000 ($679,000,000) per annum is a conservative figure. It would seem from these evidences that almost any well-organized plant, mercantile or trade establishment might find this a rich field for com-

[2] National Cash Register Company, Dayton, Ohio. It is probable that different bases of computing sickness absenteeism account for some of the difference in experiences of various companies.

mercial investment as well as for humanitarian measures.

The subject is, however, we believe, much broader than industrial surgery, first aid, and a rest room—features which are so often the only ones elaborated. It demands the application of scientific medicine and hygiene in a broad way. Remarkable results within a short time should not be expected, since much educational work in relation to physical examinations and physician's instructions in personal health as well as in plant safety and practice in hygiene is necessary. It should not be forgotten, also, that several of the leading universities[3] in the country are turning out each year a few men well equipped in the fundamentals of public health and hygiene as applied to industry, and that special academic training in the subject, as in any applied profession, is practically essential for the attainment of good results.

Summary

1. A statistical study is presented of 3,226 cases of occupational diseases reported to the Ohio State Department of Health in the five-year period ending June 30, 1925.

2. The "standard" blank used for reporting, and the laws and regulations for reporting and for compensating occupational diseases, are given with a brief historical statement.

3. A total of some 6,363 cases of occupational diseases have been officially reported in Ohio in a period of twelve years and two months ending June 30, 1925.

4. Previous publications regarding occupational disease statistics in Ohio are cited.

5. The change from the earlier method of compensating occupational diseases to that which became effective July 1, 1925, is discussed with the probable effects on the future status of occupational diseases in the state.

6. The occupational diseases statistically presented are grouped by sex, cause, compensability, and industries and trade processes. Age distributions for males are shown by graphs. Rates are not given as the numbers employed in the given industries and trades are unknown to us. There were no cases reported of glanders, phosphorus poisoning, carbon bisulphide poisoning, carbon dioxide poisoning, and caisson disease.

7. Lead poisoning is further detailed in a separate table with appropriate summary.

8. Dermatoses among rubber workers, both male and female, are discussed and a separate table is given for each sex.

9. The three major groupings of occupational diseases for males show that (1) rubber dermatitis is reported for men principally between 21 and 35 years of age, (2) lead poisoning for men principally between 21 and 40 years of age, and (3) all other occupational diseases for men principally between 17 and 50 years of age. These age groups are probably simply an index of the age dis-

[3] At the present time we would mention Harvard, Johns Hopkins, Pennsylvania, and Yale. Several other universities are also prepared for, or are actually starting, such training courses, and are only waiting for the demand to appear from the industrial field itself to encourage students, usually medical students but some others, to elect special training of the nature indicated.

tribution of those employed in the three major industrial groups given.

10. An increase in the number and range of occupational diseases is rapidly taking place.

11. A literature citation is made to the commercial significance as well as to the humanitarian aspect of the control of industrial health—apparently one of the most profitable investments in the entire industrial field.

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THE ESTIMATION OF ZINC IN BIOLOGIC MATERIAL*

LAWRENCE T. FAIRHALL, A.M., PH.D.

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

GENERAL DISCUSSION OF METHODS SUITABLE FOR THE DETERMINATION OF SMALL AMOUNTS OF ZINC

THE separation and estimation of minute amounts of zinc from biologic material is usually fraught with some uncertainty, not only because of the difficulty attendant upon the separation of the zinc itself but also because of the limitation of the methods which we possess for its estimation. A great many methods have been elaborated for the estimation of zinc but no very great attempt has been made to discover sources of error in these methods when applied to the analysis of biologic material. Furthermore, in place of investigating the range over which a particular method gives the most accurate results, the trend has usually been in the direction of applying a single method to all cases of analysis.

Zinc differs from the other commonly occurring metals not only because of the greater solubility of its salts in general but also because it forms no colored salts. It is therefore rather a difficult metal to estimate in minute amount. The methods that have usually been employed for the estimation of zinc in organic combination, etc., may be divided into the following groups:

A. Gravimetric or titrimetric processes, for relatively large amounts.

B. Turbidimetric methods, such as the sulphide and the ferrocyanide, for comparatively small amounts.

C. Colorimetric methods, for very small amounts.

Gravimetric Method

Gravimetrically zinc has usually been determined as sulphate (1) or as oxide (2) (3) when applied to the examination of animal tissue. Both of these methods lead to accurate results, especially with relatively large amounts of zinc. The amount of zinc ordinarily determined in animal tissues, however, is too small to justify their use; furthermore, they are time consuming as compared to other methods.

The ferrocyanide titration method for zinc analysis was first developed by Galletti (4) in 1864. The zinc solution acidified with acetic acid was titrated with standard potassium ferrocyanide solution, the solution being well shaken after each addition and the end point taken as that point where no further turbidity developed. In this form it was, of course, essentially inaccurate. Galletti improved the method later (5) by precipitating

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the iron with ammonium acetate and using copper salt for an indicator by testing portions on a glass rod near the end point of the titration. Fahlberg (6) used a uranium salt as an indicator. With the exception of certain refinements in technic, this is essentially the method as it exists today. Although its accuracy is limited in the lower range, it is more accurate for the larger quantities of zinc occasionally encountered than any other convenient method. In order to obtain this accuracy, however, it is necessary to insure uniform conditions in the method of titration.

Turbidimetric Method

As the need arose for estimating smaller quantities of zinc than the gravimetric or titrimetric methods would indicate, turbidimetric zinc methods were developed. These are sometimes erroneously called colorimetric methods. The basis of comparison, however, is the turbidity of a given zinc solution when treated with some precipitating substance. Zinc may be estimated in this way both as sulphide and as ferrocyanide. From neutral solution in the first case, and slightly acid in the second, zinc reacts with the appropriate reagent yielding a very slight milkiness or haziness in very low concentrations and a flocculated precipitate in high concentrations.

Sulphide.—The sulphide turbidimetric method has been applied to the determination of small amounts of zinc in water (3) and in foodstuffs (7). According to Meldrum (8), the sulphide turbidimetric method has about one-tenth the sensitivity of the ferrocyanide turbidimetric method. In addition to the facts that the hydrogen ion concentration must be carefully adjusted in order to insure complete conversion of the zinc salt to zinc sulphide and that in common with other turbidimetric methods the question of particle size must be considered, there is a further complication in connection with the use of this method—namely, the readiness with which aqueous solutions of hydrogen sulphide are oxidized. The slight turbidity thus produced may very readily be mistaken for zinc sulphide.

Ferrocyanide.—The ferrocyanide turbidimetric method is much superior to the sulphide method. Mylius (9) first used this for investigating the presence of zinc in drinking water. It has been repeatedly used with but little variation in technic for the estimation of zinc in foods (10), in water (11) (12), and in muscle, liver, urine, etc. (13).

The ferrocyanide method is a very reliable means of estimating small amounts of zinc provided the conditions to be described further on are carefully observed. No previous attempt has been made to secure the uniform working conditions for the method which are necessary for results of the greatest accuracy. For instance, with a constant amount of zinc (say, 0.1 mg.) in a series of Nessler tubes it is quite possible to obtain wide variations in the apparent amounts of zinc present by varying the acidity. Similarly, a variation in salt content will produce varying turbidities with similar amounts of zinc. By eliminating such sources of error, however, the method

can be made to yield very accurate results within the range for which it is adapted.

Colorimetric Method

The colorimetric methods which have been advocated for the detection or determination of very small amounts of zinc are in general quite inferior to the method recently developed by Lutz (14). These methods are typified by those in which a color is developed as with resorcinol in ammoniacal solution (15), or by the formation of colored lakes with dyes (16).

These methods, as well as those which depend on the formation of colored inorganic salt solution, as in the treatment of zinc ferrocyanide with bromine water (17) or in the formation of zinc cobaltocyanide ($Zn_2Co(CN)_6$) (18), are too uncertain in their action or insufficiently delicate to be of any great value. On the other hand, Lutz has placed the urobilin method for the micro-estimation of zinc upon a secure quantitative basis, so that it is possible not only to detect zinc in quantities as low as thousandths of a milligram, but also to estimate these quantities with a certainty not possible with any other means.

Ashing

Zinc is probably combined in an organic form in most of the biologic substances in which it normally occurs—*e.g.*, milk, grain, certain shellfish, etc.—and it is difficult to effect its quantitative separation without first destroying the organic material. For this reason its analytic separation has always been preceded in this laboratory by a complete ashing of the substance concerned. This part of the procedure requires particular care in order to secure reliable results. Dr. Phebe K. Thompson (19) has found that with suitable precautions the ashing may be carried to a higher temperature than is generally possible according to the opinion of those who have been concerned with the analysis of zinc. In her experiments the zinc (in the form of various salts, metallic zinc or these salts combined with zinc free organic material) was heated to various temperatures as indicated by a pyrometer for varying periods of time in an electric muffle furnace and the zinc quantitatively determined. These experiments represent the first systematic attempt at controlling zinc losses in ashing organic material. They indicate that biologic material may be ashed at a temperature of 450° with absolute safety as far as zinc is concerned. This temperature is, furthermore, a convenient one at which to ash biologic material, owing to the fact that the mineral constituents may be more readily washed from the char than if the ashing is carried out at a higher temperature.

The question of getting the zinc into an inorganic salt solution is, therefore, not difficult. None the less, the utmost care must be taken to avoid zinc losses in transfer or incomplete combustion of the material being analyzed.

PURIFICATION OF REAGENTS AND PREPARATION OF STANDARD SOLUTIONS

Certain reagents necessary for the following analytic operations need careful purification. Zinc is occa-

sionally found as a contaminating substance in hydrochloric acid and ammonium hydroxide and to a less—although significant—extent in cer-

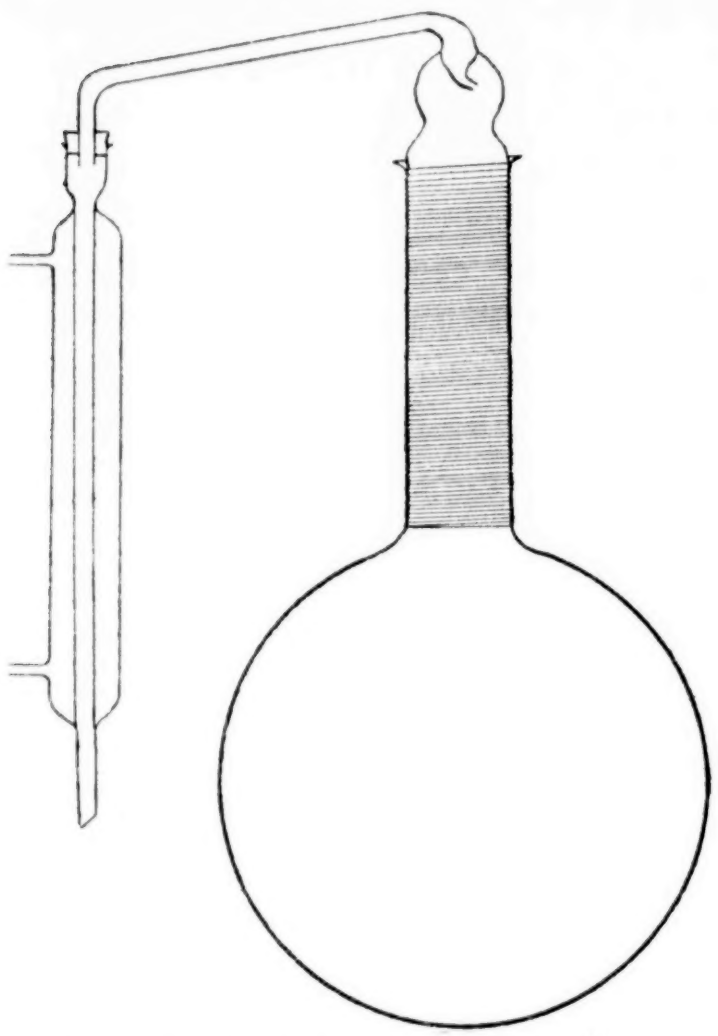

FIG. 1.—Distillation apparatus.

tain filter papers and reagents necessary for the micromethod. Nitric acid, nitrates, and ammonium acetate usually contain so little zinc that the amounts used in the course of an analysis cause no appreciable error. In case ammonium acetate contains a notable amount of zinc, it may be prepared zinc free from redistilled ammonia and acetic acid. Cupferron often contains zinc, owing to the fact that zinc dust is used as a reducing agent at one step in its synthesis. Ordinary 95 per cent. alcohol occasionally contains aldehydes which interfere with the urobilin test and, less frequently, contains zinc. The reagents may be conveniently purified and the necessary standard solution prepared in the following manner:

Hydrochloric Acid.—Dilute "C.P." concentrated hydrochloric acid with an equal volume of water and distil. The form of distillation apparatus of pyrex glass with ground-in glass connection, illustrated in Figure 1, is most convenient for this purpose, as the apparatus is very compact and requires but little attention. The capacity of the distilling bulb is 3 liters.

Ammonium Hydroxide.—Distil ordinary (0.9 sp. gr.) ammonia in the foregoing apparatus having a funnel attached to the lower end of the condenser so that the mouth of the funnel just dips beneath the surface of iced water in a large beaker.

Alcohol.—Distil ordinary 95 per cent. alcohol in the foregoing apparatus, the first portion of the distillate being discarded. Alcohol that has been used for the micromethods accumulates ammonia and water. The former may be removed by neutralization with sulphuric acid and distilling; the latter by further distillation over lime. According to Lutz (14, p. 277), this alcohol accumulates aldehydes which cause a rapid diminution in the fluorescence with urobilin and zinc salts. The first runnings of the distillate should therefore be discarded.

Potassium Ferrocyanide.—Dissolve the ordinary "C.P." potassium ferrocyanide in slightly more than its own weight of distilled water by boiling, filter the hot solution to remove the inevitable solid impurities, and crystallize the salt by rapid chilling and continuous stirring. Sepa-

rate the crystals by suction filtration and dry. For the turbidimetric estimation of zinc, use a 2 per cent. solution of this salt; for the titration method, a solution containing 3.464 gm. per liter (equivalent to about 1 mg. of zinc per cubic centimeter of solution). Potassium ferrocyanide solution should be kept in a cool, dark place in order to avoid decomposition.

Standard Zinc Chloride Solution.—The ferrocyanide solution used for titration is standardized against a solution of zinc salt of known zinc content. For this purpose, weigh accurately 1.2446 gm. of pure zinc oxide which has been ignited and cooled in a desiccator and dissolve this in pure 1:1 hydrochloric acid, using a slight excess of acid. Make the solution up to 1 liter. Zinc oxide is preferable to metallic zinc, as it is less likely to contain impurities such as iron and lead. One cubic centimeter of the foregoing solution contains exactly 1 mg. of zinc.

Uranium Acetate.—A dilute solution of this indicator is preferable to the stronger solution frequently used for titrating large amounts of zinc. Dissolve 40 gm. of uranium acetate in 800 to 900 c.c. of distilled water and allow the solution to stand for several days. A turbidity frequently develops, and some precipitation. Filter the solution and dilute to 1 liter.

Cupferron.—In the preparation of phenylhydroxylamine from nitrobenzene (a necessary step in the synthesis of cupferron—ammonium nitrosophenylhydroxylamine), zinc dust is used as a reducing agent. Consequently it is essential that the cupferron reagent be carefully tested for zinc. If zinc is present, dissolve in boiling alcohol, filter (using a Büchner funnel and suction filtration), and crystallize the cupferron from solution by rapid chilling. It is an advantage to saturate the alcohol with ammonia gas in order to reduce decomposition of the salt and to increase the yield. Usually two crystallizations suffice to remove the last trace of zinc.

Ashing Procedure

If the material to be analyzed is a liquid, slowly evaporate it to dryness in a Coors porcelain dish within a hot air oven or over a hot plate. Solids should be similarly dried in small Coors porcelain evaporating dishes. The solid material is then further heated on the hot plate till it commences to char. Transfer the dish to a muffle furnace maintained at about 450°C. A convenient ashing temperature is that point at which the furnace approaches, but does not reach, a faint red heat. The time required for ashing varies a great deal with the nature of the material. With urine, muscle, or the soft tissues, the time consumed is much longer than with feces, owing largely to the difference in content of sodium and potassium salts. Usually it is advantageous to carry the first stage of ashing only to that point where a soft carbon char is produced rather than to the coke stage which occurs later. The char is extracted with 10 to 20 c.c. of 1:1 hydrochloric acid and hot water. The filter paper and carbon residue are then dried and returned to the furnace. At this stage the carbonaceous residue should be almost completely burned. After cooling, the ash is extracted as before and the small amount of charcoal

again returned to the furnace. The careful addition of a drop or two of nitric acid will usually insure the complete combustion of the carbon at this point. It is essential that the final residue of carbon be completely consumed, otherwise some zinc will be lost here. The volume of solution representing the combined extracts should be about 100 c.c. for a 100-gm. sample of muscle.

Analytic Separation of the Zinc

The mode of analysis for zinc in biologic material is determined largely by the amount of zinc present and therefore depends on the judgment of the analyst. There is no one method of analysis that will apply with strict accuracy to the various amounts of zinc that may be encountered. A certain amount of experience is necessary, therefore, with the various methods detailed below and with the types of material for analysis, in order to insure accurate results. The analytic procedure has accordingly been divided into three groups. With amounts of zinc above 1 mg., it is more reliable to depend on titration with potassium ferrocyanide (I). The ferrocyanide turbidimetric method (II) is most accurate for amounts ranging from 0.05 to 2 mg., and Lutz' microchemical method (III) for amounts ranging from 0.01 mg. up.

Whatever method of estimation is applied, the general scheme of separating zinc from the other substances present in the ash is the same. It is essential that all interfering substances such as iron, copper, calcium, and salts of the alkalies be successively eliminated before the final estimation of the zinc. This is not particularly difficult, although it lengthens the method of analysis. Unless these salts are removed, however, they interfere markedly with the definition of quantities of zinc present. For instance, traces of iron and copper interfere seriously with the end point in titration; sodium, potassium, and calcium salts lead to high values in estimating zinc by the turbidimetric method; and calcium and magnesium lead to low values in the microchemical method. It is, therefore, necessary that the zinc be freed from all these substances before the final step in analysis is undertaken. The following analytic separation of zinc applies with only slight modification to all three methods of analysis.

The acid solution containing the dissolved ash should have a volume of about 75 c.c. containing 10 to 15 c.c. of 1:1 hydrochloric acid. If the volume is much greater than this, gently boil to a volume of 75 c.c. and cool. Add to the cold solution an excess of cupferron (ammonium nitrosophenylhydroxylamine) dissolved in water. This precipitates the iron and part of the copper ordinarily present. Iron forms a reddish and copper a dirty gray precipitate. The iron is completely precipitated when white crystals of the cupferron reagent are noticed on further addition of the reagent. The solution containing precipitated iron is difficult to filter at first, owing to its colloidal form. It is necessary, therefore, to shake the solution until the colloid is coagulated. Repeated shaking will coagulate the colloid and the solution filters readily giving a clear, slightly

greenish-colored filtrate. The presence of a great excess of acid—particularly in warm solution—causes partial decomposition of the cupferron and darkening which obscures the end point on subsequent neutralization. The precipitation must occur in cold solution and with the degree of acidity indicated. Owing to the fact that the precipitation occurs in acid solution, no loss of zinc is experienced at this point. The filtrate, if strongly acid, is partly neutralized with sodium hydroxide and, finally, ammonium acetate is added until the free hydrochloric acid is completely replaced by acetic acid as indicated by methyl orange. To this solution is then added 5 mg. of copper as copper nitrate, and the solution is diluted to a volume of 150 c.c. and saturated with hydrogen sulphide gas. The copper serves as an entrainer, since the zinc is completely removed from solution as sulphide by adsorption on the copper sulphide. In the absence of copper, zinc, if present in small amount, will be very incompletely precipitated. In addition to these sulphides, there is precipitation of decomposition products of the cupferron which form on treatment with hydrogen sulphide. The sulphide precipitate is well washed with cold water and finally with hot alcohol in order to remove the organic material. The funnels are then returned to their original flasks, the precipitates washed once with hot water and then treated alternately with concentrated nitric acid and hot water till completely dissolved. Evaporate the nitric acid solution to dryness, add 1 c.c. of sulphuric acid (1:2) and 2 c.c. of nitric acid, and heat till the remaining traces of organic material have been completely oxidized and the excess of sulphuric acid fumed off. Dissolve the residue in 5 c.c. of 1:1 hydrochloric acid, cool, add 25 c.c. of water, and saturate the cold solution with hydrogen sulphide gas. Filter. Evaporate the filtrate to dryness. In case there is much calcium present, it is nearly impossible to obtain calcium free zinc at this point. As traces of calcium interfere with the micromethod of zinc estimation, it will be found advisable as a routine procedure to reprecipitate the zinc and copper sulphides in all cases where this method is applied. In the analysis of bones for zinc, calcium phosphate will occasionally slowly precipitate from the cold solution saturated with hydrogen sulphide gas. It is therefore advisable to filter at once after saturation, wash and dissolve the sulphide precipitate, and reprecipitate the zinc and copper.

Instead of removing iron by the cupferron method, the method used by Weitzel (12) may be employed. Oxidize the iron to ferric iron by boiling the acid solution of ash with bromine water, cool and precipitate the iron as ferric phosphate by the addition of a small amount of sodium phosphate and neutralization with ammonium acetate. Allow the precipitate to stand, filter, dissolve the precipitate in hydrochloric acid, reoxidize and reprecipitate. Combine this filtrate with the original filtrate from the phosphate precipitation. This reprecipitation is necessary since part of the zinc is carried down with the first precipitate of ferric phosphate. This method involves much more time than the cupferron process and the

filtration is tedious owing to the slimy character of the phosphate precipitate.

The procedure from this point is exactly similar to that involved following the cupferron precipitation of iron excepting that oxidation with nitric and sulphuric acids is rarely necessary. In the final step, a solution of zinc chloride is obtained which must be evaporated to dryness and which for convenience may be termed the *zinc residue.*

I. Titration Method

Zinc chloride reacts in hydrochloric acid solution with potassium ferrocyanide giving a precipitate of zinc ferrocyanide. By using an indicator which will react with the excess of potassium ferrocyanide, the amount of ferrocyanide required by the zinc may be determined. The indicator which has been found to be most suitable at low concentrations of zinc is uranium acetate. It is used externally and the titration followed on a porcelain spot plate. Insufficient practice with the end point will invariably lead to overtitration. Furthermore, the temperature at which the titration is conducted is important and the end point is taken as that point at which the faintest brownish coloration appears after the lapse of at least one minute. An indicator allowance must be made for the excess of ferrocyanide required to react with the indicator after all the zinc is combined as ferrocyanide. The successful application of this method of zinc estimation lies, therefore, in the adoption of uniform conditions throughout. Temperature and concentration are factors that must not be varied if the greatest accuracy is to be obtained. Once having adopted, however, a standardized procedure in the standardization of the ferrocyanide solution used, this same procedure applied to the determination of zinc in amounts above 1 mg. will give accurate results.

Procedure.—The titration is carried through in the following manner: Prepare a solution of potassium ferrocyanide having an equivalent value of 1 mg. of zinc per cubic centimeter (3.464 gm. of crystalline potassium ferrocyanide—$K_4Fe(CN)_6 \cdot 3H_2O$—per liter of solution). The solution should stand for twenty-four to forty-eight hours and the slight residue should be filtered out. If prepared from pure salt and stored in the dark, the solution is less apt to change. Its zinc value is obtained by the analysis of a standard zinc chloride solution. Evaporate known amounts of a standard zinc chloride solution to dryness, add exactly 5 c.c. of 1:1 hydrochloric acid and 20 c.c. of water. For small amounts of zinc, reduce the volumes to 1 to 2 c.c. of hydrochloric acid and 4 to 8 c.c. of water. Heat to the boiling point and titrate with ferrocyanide, using a spot plate and uranium acetate as an external indicator. The solution should not be allowed to become cooled appreciably during the titration, and the lapse of time required for color development in the indicator should be uniform. Blank experiments using the same volumes of acid and of water, and the same temperature of titration will establish the indicator allowance which must be deducted. This allowance will vary slowly with the volume of solution, and if very

much ferrocyanide is used, as with larger quantities of zinc, an estimation of the indicator allowance for that volume of solution must be made. With uniform conditions as outlined above, this method will give accurate results for the relatively gross amounts of zinc occasionally encountered in biologic material.

II. Turbidimetric Method

For amounts of zinc which lie between those conveniently determined by titration and the extremely small amounts determinable by Lutz' method—*i.e.*, a range of from 0.05 mg. to 2 mg.—the turbidimetric method is most satisfactory. This method, first proposed by Mylius (9), consists in comparing the turbidity produced by potassium ferrocyanide with a given amount of zinc solution, with that similarly produced with various amounts of a standard solution of zinc. Nessler tubes having optical glass bottoms and supporting a column of liquid of approximately 17 by 210 mm. are best suited for comparison, and some care must be used in selecting good light and a dead black background for best results. Experience has shown that the most suitable range of standards is from 0.05 mg. of zinc to 0.55 mg. and that especially accurate readings can be made over the range, 0.15 to 0.35 mg. of zinc. The success of this method, as with the turbidimetric method, depends on absolute uniformity of conditions. Variations in salt content and in acid concentration cause large variation in particle size and, hence, in the degree of turbidity with a given amount of zinc; sunlight or oxidation produce blue ferric ferrocyanide which obscures the readings. With the modification noted in detail in the following discussion these factors may readily be controlled.

Procedure.—Dissolve the *zinc chloride residue* carefully in 10 c.c. of N/10 sulphuric acid, evaporate to dryness, and carefully fume off the excess of acid. Dissolve the residue of zinc sulphate in 5 c.c. of N/10 sulphuric acid and make the volume up to exactly 25 c. c. with distilled water in a volumetric flask. Transfer various known amounts of this solution to Nessler tubes and arrange a set of zinc sulphate standards in Nessler tubes ranging from 0.05 mg. to 0.55 mg. of zinc. Zinc sulphate is used as the standard, as it gives somewhat sharper readings than zinc chloride. In order to adjust the degree of salt concentration add phenolphthalein indicator to each tube and determine the amount of N/10 potassium hydroxide required for the most acid tube—usually the unknown—and then add a similar amount of alkali to every other tube in the set. Titrate each tube to neutrality with N/10 sulphuric acid and add 1 drop excess of sulphuric acid. In this way each tube will have a similar salt content and degree of acidity. Add 1 c.c. of a cold saturated solution of sulphurous acid to each tube, dilute to about 45 c.c., add 1 c.c. of 2 per cent. (freshly prepared) potassium ferrocyanide solution, dilute to the mark, and shake thoroughly. The sulphurous acid prevents any oxidation and hence any greenish coloration that would be likely to interfere with the readings. Set the tubes aside in a dark place for twenty minutes in order

to allow the full turbidity to develop. In comparing the turbidities, avoid direct sunlight and make the readings against a dull black background.

III. Micromethod

For quantities of zinc below 0.05 mg. the only accurate procedure is that developed by Lutz (14). This method is discussed in detail in Lutz' original article and will be but briefly summarized here. Inasmuch as the amounts of tissue that are to be analyzed by this method are frequently very small, and in order to reduce further the chance of contamination, most of the operation of ashing, extraction, precipitation, etc., should be carried out in very small dishes and flasks.

Procedure.—The iron may be removed by the phosphate precipitation method with practically as much facility as by the cupferron method since but small volumes of solution are involved. It is essential, however, to be especially careful to wash out salts which may be mechanically carried along with the zinc precipitate so that the *zinc residue* finally obtained is free from traces of calcium, magnesium, copper, or the alkali metals. Use nothing but #40 Whatman filter paper throughout, as other grades of filter paper contain appreciable amounts of zinc. The detection of zinc by this method is facilitated by using 30 to 40 mm. filter paper and microfunnels and microflasks. Evaporate the zinc chloride residue carefully to the point at which the last drop of liquid is vaporized by breathing on it. This will leave sufficient acid in the residue to insure complete solution. If evaporation is continued to dryness on a hot plate a partly insoluble residue is obtained necessitating resolution in acid and reevaporation. Careful evaporation, however, will yield a residue completely soluble in water. Adjust the volume accurately to 5 or 10 c.c. and use convenient aliquot parts of this for the actual estimation of the zinc. Into Nessler tubes similar to those used for the turbidimetric method, introduce 15 to 20 c.c. of redistilled 95 per cent. alcohol, add a known portion of the zinc solution and about 20 c.c. more of alcohol, mix the contents of the tubes, add 1 to 2 c.c. of urobilin solution, adjust to the 50 c.c. mark, add 1 drop of ammonium hydroxide and again carefully shake the tubes. If illuminated by a carbon or iron arc lamp against a dark background a greenish fluorescence will be noted. The depth of the fluorescence is proportional to the amount of zinc and is strikingly apparent in amounts as low as 0.001 to 0.002 mg. of zinc. With the aid of standard tubes containing known amounts of zinc, it is, therefore, possible to estimate small quantities of zinc very closely.

Conclusion

The three procedures give an accurate means of estimating zinc in biologic material. Owing to the wide and unequal distribution of zinc in such material, no one method suffices for the whole range, but with experience in the type of material analyzed the methods as modified give results more accurate within the range for which they are particularly suited than a host of methods described in the literature.

The methods as developed have been repeatedly checked. For this purpose, known quantities of zinc were added to material such as starch or other organic material containing negligible amounts of zinc and the zinc recovered by one or the other of the processes. Frequently use was made of an inorganic salt solution free from zinc. This solution was so adjusted that 15 c.c. contained the amounts of salts normally excreted by an average man per day. To this "artificial ash," known quantities of zinc were added. The interfering metals were then successively eliminated and the zinc estimated. Recoveries of zinc made in this manner indicate that an accuracy within 5 per cent. of the amount actually present may be expected in each range of zinc concentration. It is possible, therefore, to estimate satisfactorily inbiologic material quantities of zinc ranging in amount from 0.002 mg. to 50 mg.

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THE NORMAL OCCURRENCE OF ZINC IN BIOLOGIC MATERIALS: A REVIEW OF THE LITERATURE, AND A STUDY OF THE NORMAL DISTRIBUTION OF ZINC IN THE RAT, CAT, AND MAN*

ROBERT E. LUTZ, PH.D.

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

I. INTRODUCTION

A REVIEW of the literature on the normal biologic occurrence of zinc reveals the striking fact that zinc is a common, if not an almost universal, constituent of plant and animal materials. A very large amount of work on the detection of zinc has been reported and most of the results, especially those obtained in recent years by means of the more refined analytic methods available, have been positive. Zinc has been found, furthermore, not in mere traces, but in relatively large amounts as compared to other heavy metals, and in concentrations of approximately the same order of magnitude. A few authors, who have noted the widespread normal occurrence of zinc (1) (2) and have speculated upon its possible biologic significance (Bertrand and his associates and Delezenne, whose several articles are referred to more specifically later, Piccinini (1), etc.), have drawn the definite conclusion that this metal occurs as a universal mineral component of plants and animals, but the mass of evidence in favor of this view has not been generally recognized.

Although this hypothesis of the universality of zinc is supported by a large number of positive experimental results, one cannot ignore the many contradictions to be found in the literature. Some of the early investigators, as a result of the crudeness of their analytic methods and of the difficulty of detecting zinc, especially in the presence of the excesses of other inorganic salts which invariably occur in biologic material, failed to detect this metal in many materials which are now known to contain very definite and considerable amounts of it. Analytic methods and technic, though still far from perfect, have been improved tremendously in the last few years, but in spite of this there are discrepancies in many of the more recent results on different samples of the same materials. These discrepancies may, in all probability, be accounted for mainly by the lack of sensitive, reliable, analytic methods, and the failure to standardize adequately the analytic procedure used, rather than by the existence of great variation in the zinc concentrations of biologic materials of the same kind.

Because of the wide occurrence of zinc and a very possible functional importance of the metal in vegetable

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and animal life processes, it is of great importance to examine carefully the hypothesis that the occurrence of zinc in biologic materials is not accidental, but is normal and universal, and to determine whether or not zinc occurs in any given organ or biologic tissue in concentrations which are approximately constant under normal conditions. This problem, unlike that involved in the determination of the actual biologic function of zinc, is purely empirical and can be attacked and solved analytically. In this paper, I shall examine this hypothesis, first, by means of a critical survey of the literature and summary of the large amount of analytic data which has been reported; and second, by means of a complete analytic investigation of the normal distribution of zinc in the rat, cat, and man, with particular reference to the universal occurrence of zinc in all the tissues, and the constancy of the concentration of zinc in different samples of the same tissues taken from different individuals.

The question of the possible biologic function of zinc and the physiologic processes by which an animal handles, stores, and eliminates zinc is beyond the scope of this study, but will be considered in a paper which will be published later as part of a general investigation of the hygiene of zinc being carried on in the Department of Physiology of the Harvard School of Public Health. In experimental work in this field, determination of the normal distribution of zinc in the animals studied is of great importance, because, in compiling and interpreting results of experiments on the distribution, storage, or elimination and on the effects of zinc abnormally introduced into an animal, one must either apply corrections for the normal occurrence of the metal in the tissues studied, or else show that the amounts of zinc normally present therein are negligible as compared to the amounts found under the special conditions of the experiment.[1] The data available in the literature are not sufficiently extensive or reliable to furnish complete quantitative values for the amounts of zinc normally distributed in the particular animals chosen for study in our general investigation of zinc—namely, the rat, cat, and man. The data compiled in this paper, therefore, assume considerable importance as a standard for comparison with the figures obtained in animals into which zinc has been introduced abnormally, and for comparison with those obtained in men exposed to zinc in industry.

The important objects of this paper are then, first, to determine whether or not zinc occurs universally and in constant concentrations throughout biologic tissues and materials, and, second, to furnish normal standards of reference for experimental work.

[1] The great majority of investigators studying the physiologic action of zinc have failed to take into account its normal occurrence in animals. The paper of Salant, Rieger, and Treuthardt (3) may be cited as an example. Because of the failure of these authors to account for the amounts of zinc normally present in their animals, the reader is unable to accept as definite experimental evidence any of the results reported on the recovery of zinc ingested or injected. Many other investigations reported in the literature and purporting to show the disposal and distribution of doses of zinc administered in different ways are without value or significance because the authors failed to recognize this important consideration.

II. Review of the Literature

A. Sources of Zinc

The zinc present in soils, sea water, and materials comprising the ocean bottom must be the ultimate sources of zinc found in plants and animals. The presence of zinc in small concentrations in sea water has been demonstrated by Dieulafait (4) and Bodansky (5), and the metal has often been found in spring and mineral waters, occurring in widely varying amounts from 0.03 to 8 mg. per kilogram[2] (6). Materials dredged from the bottom of the ocean contain large amounts of zinc, according to Hiltner and Wichmann (7), who analyzed débris and vegetable matter obtained from oyster beds and found from 47 to 108 mg. of zinc per kilogram. Soils in general contain small amounts of zinc. In many localities, however, the concentration is very high, owing to the occurrence of large amounts of such minerals as calamine. Montanari (8), who examined many samples of soils and found from 7 to 11 mg. of zinc per kilogram of anhydrous material, discovered also that wood ashes contain considerable amounts of zinc, a fact which shows that trees absorb the metal from the soil. All plant and vegetable life, then, comes in contact with a tremendous reservoir of zinc in which, however, the concentrations are often extremely small.

B. Occurrence of Zinc in Plant Life

Plants and Trees.—The presence of zinc in plant materials immediately presents a series of problems concerning the amounts of zinc absorbed, the extent and distribution in the organs, and also the significance and function, if any, of the metal in connection with plant life. Certain plants and trees growing on zinciferous soils (those containing relatively large amounts of zinc) have been analyzed, and, as predicted by the investigators, Bellingrodt,[3] Risse,[3] and Fricke (9), yielded considerable amounts of the metal on analysis (from approximately 1 to 13 per cent. of the ash). Many trees and plants growing on soils not particularly rich in zinc (non-zinciferous) have also proved on analysis to contain varying but considerable amounts, and zinc has been found to be present (10) (11) in all organs of the phanerogamous plants, molds, algae, and many trees, particularly the conifers. In the large number of trees listed by Javillier (10), the zinc content of those grown on non-zinciferous soils, as determined by his analyses, ranged from 2 to 135 mg. of zinc per kilogram (dry weight). Failure to detect zinc in plants and other vegetation has occurred so rarely that one is inclined to believe that in these few isolated cases zinc was probably present in small concentrations but escaped detection because the methods of analysis used were not sufficiently sensitive.

Tubers.—Lechartier and Bellamy (12) first detected zinc in food products, including certain grains and tubers. Since then many other investigators have analyzed various foods, and the

[2] No data will be included or discussed in this paper, on the rather common, though abnormal, occurrence of zinc in drinking waters or in foods, where the zinc has been introduced artificially through the use of the metal in piping, galvanized wares, etc.

[3] Quoted from Sacher (2) who published a very thorough review of the physiologic action of zinc salts.

presence of zinc, often in considerable amounts, has invariably been shown. Zinc is absent, to be sure, in some food products, such as beet sugar (13). Usually, however, the metal is present in the raw materials from which the food product is derived, and zinc is, in fact, present in beet roots (12). Sugar is a soluble, highly purified substance, and it is not surprising, in view of the recrystallization and filtration through bone black, that zinc is absent in the final product, though present in the beet itself. Soluble starch, on the other hand, despite elaborate purification processes, still contains appreciable amounts of zinc (3 mg. per kilogram[4]) (14). Potato starch has been shown to contain 1 mg. of zinc per kilogram (15) (16); casein, 4 mg.; and potatoes themselves, 2.3 mg. (13) (6). One commercial grade of cooking cornstarch has been found to contain as high as 35 mg. of zinc per kilogram (14). All filter papers, even those washed with acids, contain appreciable amounts of zinc which can be readily estimated by means of urobilin (14) (6). These facts indicate that zinc is bound up in the very structure of plant cells.

Grains.—Zinc has been found in the large number of the cereal grains which have been investigated, in amounts varying from approximately 14 to 84 mg. per kilogram (17). Among the materials which have been studied are corn (25.2 mg. per kilogram), wheats (26 to 84.8 mg.), barley (26.7 mg.), oats (31.7 to 49.3 mg.), rye (17.1 mg.), maize, rice (14.6 mg.), soy bean, haricots, white beans, and dry peas (34.5 mg.). Furthermore, it has been shown that in grains the zinc is concentrated in the bran; wheat bran, for example, containing 139.2 mg. per kilogram. In corn, however, the zinc is concentrated in the germ (17). Flour contains only small amounts of zinc (0.8 mg. per kilogram); graham flour, 3.8 mg. per kilogram (14); and Wheatena, a typical cereal product which contains a moderate amount of the bran and, therefore, more of the mineral constituents of the grain, contains as high as 31 mg. of zinc per kilogram. The value of the brans in nutrition, because of the high mineral and vitamin content, has been generally recognized, and zinc must now be included among the important mineral constituents, although its rôle is as yet obscure.

Miscellaneous.—Analyses have been made on such miscellaneous food products as coconut oil (12.5 to 40 mg. of zinc per kilogram) (18), yeast (414.8 mg. per kilogram) (17), gelatin (27.4 mg.), malt (11 mg.), oranges (1 mg.),[5] fresh and dried vegetables, carrots (4.9 mg.) (17), spinach (9 mg.) (8), and dried apples (19), which contain small amounts of zinc even when dried on wooden racks instead of on the usual zinc trays. Lettuce and cabbage, which we have used as green foods for the rats in our general zinc investigation, have been found on analysis in this laboratory to contain, respectively, 1.3 mg. and 1.98 mg. of zinc per kilogram.

Zinc has been found, then, in all plant and vegetable materials that have been analyzed, and in amounts often comparable to those in which iron occurs.

[4] Unless the condition of the sample analyzed be specified, the term "milligrams per kilogram" will refer to milligrams per kilogram of normal, hydrous material.

[5] Unpublished data obtained by Dr. L. T. Fairhall.

It has been suggested, because of this fact, that zinc, as a normal constituent, is necessary to plant life and that it probably plays some important but as yet unknown part in vegetable metabolism.

C. Occurrence of Zinc in Animal Life

Marine Organisms.—Quite extensive investigations have been made on the occurrence of zinc in marine animals. The zinc content of sea water is extremely low, varying from 0.002 mg. per kilogram in the Mediterranean (4) to 0.0073 mg. per kilogram in the Gulf of Mexico (5). The presence of zinc in sea water off New York has been questioned (7) but, since relatively small samples of the water were analyzed, the absence of minute amounts of zinc is questionable. The materials comprising the floor of the ocean, as has already been indicated, contain relatively large amounts of zinc, and these materials, together with the zinc in sea water, constitute the ultimate source of the metal in the organisms studied.

An experimental study of the molluscs, carried out by Bradley and Mendel (20) (21), has demonstrated the normal occurrence of zinc, together with iron and copper, in a large variety of marine organisms. Determinations were made of the zinc content of the blood, liver, and tissue of the Sycotypus canaliculatus. A study has been made by Phillips (22) of marine organisms which were taken from the vicinity of the Tortugas Islands where there was no possibility of any abnormal contamination of the water in which these organisms lived. With the possible exception of the blood of the crayfish,[6] zinc, together with iron, magnesium, and copper, was found to be universally present in the organisms studied—*i.e.*, conch, crayfish, sea cat, tunicate. The amounts of zinc found varied from 24 to 532 mg. per kilogram of material dried at 110°C.; the organs analyzed included the muscle, liver, blood, and total organism. The amounts of zinc found by Hiltner and Wichmann (7) in oysters were surprisingly large, although varying from 26 to 2,298 mg. per kilogram. The average figure was far higher than any of the results obtained in the analyses of Tortugas marine organisms (22).

Data on the zinc content of many marine organisms have been given by Bodansky (5) in a list including some twenty different species. Oysters gave high results, 188.5 to 341 mg. per kilogram; sea catfish, 97.5 to 149.1 mg.; clams, 77 mg.; while the other individuals in the list varied in zinc content from 2.5 to 22.8 mg.

Several other investigators have studied the zinc content of the molluscs. Portuguese oysters have been found by Bertrand and Vladesco

[6] Failure to detect zinc in materials of plant and animal nature has been rare. In those cases—several of which have been indicated—one may assume that zinc is present, but in quantities below the limits that can be detected by the analytic method used. I shall make this assumption, and base it on the facts, first, that the analytic methods used were not adequate to cope with such minute amounts of zinc as were (I assume) present; and second, that no exceptions to the generalization assumed have been found in any of the results obtained with the urobilin method of analysis (14) given in the experimental part of this work. There may be exceptions to this generalization, but before one should recognize any specific case the most sensitive possible method, and accurate, standardized technic should be employed to yield negative results.

(23) to contain 35.9 to 131.1 mg. of zinc per kilogram, and these authors in a series of analyses carried out on a number of marine organisms found a normal occurrence of zinc varying in the different species and tissues from 0.4 to 9.3 mg. per kilogram.

Delezenne (24) in his paper reported results of analyses of various species of marine organisms including the molluscs, crustaceans, and certain fish, and he also detected zinc in certain insects and amphibians.

An attempt has been made to compare the foregoing results with the amounts of zinc occurring in marine animals of the Pacific coast of the United States, and it has been found by Hazel Severy (25) that California oysters, while high in zinc as compared to other marine animals, contain much less of the metal than do eastern oysters. In Severy's investigation zinc was found in all of the marine animals studied and averaged in concentration 22.8 mg. per kilogram. (In the article as originally published, an average of 4.25 was given—an erroneous figure which was later corrected to 22.8 in a publisher's note.)

The distribution of zinc in the organs of two species of fish (26) has been studied in detail, although no conclusions were drawn from the results. Mention might be made at this point of the occurrence of zinc in cod liver oil (23 to 40 mg. per kilogram) (18). Bertrand and Vladesco (27) studied the concentrations of zinc in the tissues of the herring and attempted to correlate variation of the zinc concentration in the reproductive organs with the periods of sexual activity. Their correlations were not conclusive, although they showed quite clearly that zinc is invariably present and is an important mineral constituent of all tissues of this species of fish. Bertrand (28) also attempted to show, but with indifferent success, that the concentration of zinc in the total organism was at a maximum in the young fish and reported in this connection total concentrations of 18 to 142 mg. per kilogram in three species.

A study of the distribution of zinc in two marine mammals, the sea lion and the whale (25), was made to determine to what extent zinc ingested in foods (marine organisms) accumulates in these animals. Zinc was found in all tissues studied. It is apparent from this work, however, that the mammals do not store large amounts of zinc but are able to excrete the metal with comparative ease.

Reptiles.—A remarkably high concentration of zinc has been reported by Delezenne (24) and by de Gramont (29) in serpent venom. A number of experiments were carried out by Delezenne on the occurrence of zinc in the blood and organs of certain reptiles, and concentrations of zinc were found which ranged from 30 to 53 mg. per kilogram. In the ash of a number of serpent venoms, high concentrations of zinc were reported, varying from 1.1 to 5.6 gm. per kilogram, and the concentrations were roughly in the order of and proportional to the toxicity of the venom.

Fowl.—Analyses of the organs of the duck and the hen have been made by Delezenne (24), who reported the presence of zinc in the blood (20 to 23 mg. per kilogram), liver (67 to 76 mg.), and muscle (27 mg.).

Mention should be made here of the normal occurrence of zinc in eggs.

The results of the various investigators indicate conclusively that the zinc, invariably found in egg products (30), is concentrated in the yolk and is present in minute amounts in the white and in the shell. Varying analytic results have been obtained for the yolk, Wolff (18) finding approximately 4 mg. per kilogram as one extreme, while Birckner (17) and Bertrand and Vladesco (31) found as high as 44.5 to 56.7 mg. per kilogram. The results which I reported in a previous paper averaged 26 mg. per kilogram (14). It is evident then that zinc is a normal constituent of the yolk of hens' eggs, but is present in the white of egg in relatively small concentrations.

Animal Tissues and Organs.—The distribution of zinc in animal tissues and organs is of particular interest and a general discussion will be given of the extent to which the study of this distribution has been carried. Brief mention should first be made, however, of the normal occurrence of zinc in the *milk* of various animals. Analyses of cows' milk have been reported ranging from 2 to 5.4 mg. per kilogram (13) (17) but averaging about 4 to 4.5 mg. during the main period of lactation. The analytic results of various investigators agree fairly closely. Rost found 2.3 mg. of zinc per kilogram in goats' milk and 1.3 to 1.4 mg. in human milk (see also Giaya (32)), but Birckner reported a much higher concentration of zinc in human milk (5.6 to 13.7 mg.). Despite the variation in results, one cannot but recognize that zinc is an integral mineral constituent of milk.

The presence of zinc in animal tissues was first established by Lechartier and Bellamy (12) who identified the metal in muscle and liver of man and cattle. Raoult and Breton (33), in the same year (1877), reported results of analyses confirming the presence of relatively large amounts of zinc in human liver. To the information of these men has been added a very large amount of data, much of which is contradictory. The results which lend themselves to tabulation have been assembled in Table 1. It is probable that many of the figures reported are unreliable, owing to the use of poor analytic methods and, in a lesser degree, to the nature of the materials studied, since in some cases considerable variation in the concentration of zinc in different samples of the same biologic material is observed. This latter factor appears to some extent in the analyses of liver—a fact which one might expect in view of the nature and function of this organ. But great variation should not occur in such tissues as blood, muscle, bone, and hair.

In the *blood* stream of all animals examined, zinc has been found in a small but apparently very definite concentration. The data in the literature are, however, far from complete. According to Rost (6) and Weitzel (34), probably the most reliable of the workers in this field, cows' blood contains from 5.3 to 5.7 mg. of zinc per kilogram, while the results obtained by myself (14) for the same material averaged 5.1 mg. per kilogram. The bloods of several other animals have been investigated by Delezenne (24) but his results are invariably three to four times as high as the values given above for cows' blood, and as my results for rat, cat, and human blood. Among the ani-

mals studied by Delezenne were man, cow, dog, horse, and rabbit, and the concentrations of zinc ranged from 15.1 to 26.1 mg. per kilogram, figures which are far higher than those obtained by Rost, Weitzel, Fairhall (unpublished data), and myself. Fairhall has recently found dogs' blood to contain 4 to 5 mg. per kilogram. Miss Severy (25) has reported that the blood of the sea lion contains 1 mg. of zinc per kilogram. The blood stream, then, carries a small but definite amount of zinc.

It is of interest, perhaps, to consider the quantitative relation of zinc to the other mineral constituents in blood. In 1 kg. of cows' blood, for example, is found approximately 5 mg. of zinc, 22 mg. of magnesium, 49 mg. of calcium, 338 mg. of potassium, and 387 mg. of iron.[7]

The average diet of animals contains the zinc which is ingested by the animal, and which, in order to enter the organism, must pass through the walls of the *stomach* and *intestine*. The sole analyses reported in the literature on man (6) give a concentration of 7.5 mg. per kilogram in the walls of the large intestine, a result which has been confirmed in this investigation. In rabbits, Bertrand (31) reported, on the basis of his analyses of the stomach, small and large intestine, and intestinal and stomach contents, a distribution of zinc in concentrations ranging from 18 to 24 mg. per kilogram.

From the point of view of their zinc content, the *livers* of various animals have been studied probably more than any other organ or tissue, but without consistent results. There seems to be no doubt that the concentration of zinc in the liver depends on the particular conditions, especially with regard to diet, under which the animal lived. The first figures which were reported on human liver, by Raoult and Breton (33), varied from 10 to 76 mg. of zinc per kilogram; Rost and Weitzel (6) (36) found from 39 to 145 mg. Mention should be made in this connection of the following authors: Bertrand and Vladesco (31), Delezenne (24), Giaya (32), Ghigliotto (37), Fleck (38), van Itallie and van Eck (39), and Cristol (40). This last author found 115 mg. of zinc per kilogram in human liver. It is probable that the variations in results obtained by different investigators are due in part to inadequate analytic methods, but there are, however, in this case such large variations, observed by the most reliable of the investigators, that one is forced to believe that the normal concentration of zinc in the human liver is variable, a fact which indicates strongly that the amounts of zinc found in the liver, above a certain minimum, are transitory and dependent on conditions and diet. It is also true that the average concentration of zinc in the liver is considerably higher than in other organs and soft tissues.

In the livers of other animals, a similar variation in the normal zinc content has been found. In cattle, the zinc concentration reported, especially by Rost and Weitzel (6), ranges from 35.5 to 83.3 mg. per kilogram; and in other animals studied, including the dog, horse, rabbit, and sheep, there has been observed the same high and variable concentration of zinc. The comparison between the

[7] Calculated from data given in Abderhalden (35, p. 554).

livers of different animals reveals no striking differences and, in general, the very high concentration of zinc reported in many cases is always compensated by other figures for the liver of the same animal which are very low. Miss Severy (25), who studied certain marine organisms, including particularly the whale and the sea lion, found that the concentrations of zinc in the livers (40 and 48.4 mg. per kilogram, respectively) were higher than in other parts of the body, with the exception of muscle, and were comparable to the concentrations of zinc reported in the livers of land animals. The *bile*, in general, has been found to contain minute amounts, if any, of zinc, but Miss Severy reported 2.4 mg. of zinc per kilogram in the bile of the sea lion. The list of animals included in these investigations is a large and varied one, and the constant occurrence of zinc in the liver is significant.

In the *kidneys*, the concentration of zinc, while lower than in the liver, seems to be somewhat more constant, although there are only a few results (listed in Table 1) on which to base a conclusion. Bertrand (31) found 19 mg. of zinc per kilogram in the kidneys of sheep, as against 39 mg. per kilogram in the liver. In human kidney, Cristol (40) found 68 mg. per kilogram. In general, however, there have been no very wide range of results and no exceptionally high analyses reported for the concentration of zinc in the kidneys.

The *pancreas* has been analyzed, in the case of the ox and the dog, by Delezenne (24) who found high concentrations of the metal, 23 and 48 mg. of zinc per kilogram, respectively. Among the many organs examined for zinc, mention should be made of the *spleen* and *lungs* in which this metal has been found regularly and in fairly constant concentrations in cattle, dog, rabbit, and horse (Delezenne, Bertrand and Vladesco, Rost and Weitzel). It is important to note that in general the results of Delezenne and also of Bertrand are constantly much higher than the results of Rost and Weitzel on similar materials. For the lungs of the horse, Rost and Weitzel (6) found 11 mg. per kilogram as against 39 mg. reported by Bertrand and Vladesco (41). The spleen of the sea lion, according to Miss Severy (25), contains from 10 to 12 mg. of zinc per kilogram, and Rost and Weitzel found 13.5 mg. per kilogram in the spleen of an ox, while Cristol (40) reported 42 mg. per kilogram in human spleen and Delezenne 23 to 29 mg. per kilogram in the spleen of a dog. One can draw only one conclusion: that zinc constantly occurs in these organs. A definite quantitative estimation of the zinc concentrations cannot be given until more elaborate and carefully controlled experiments have been conducted. The results of my own investigation, however, indicate that the data of Rost and Weitzel are in all probability the most reliable.

High zinc concentrations have been reported in certain glandular organs, but these results (those of Delezenne and of Bertrand) are not convincing because the authors have obtained rather questionably high results in other connections. Delezenne reported that the *thymus* of the ox contained 73 to 99 mg. of zinc per kilogram and the *thyroid* of the horse only 17 mg. per kilogram. For normal *mammary*

gland Taverne (44) reported the finding of 3.74 mg. of zinc per kilogram. Bertrand and Vladesco, who investigated particularly the reproductive organs, obtained very high results in many cases, which led them to believe that the metal plays an important part in reproduction (27). These authors reported considerable variation in the concentration of zinc in the various glands and tissues that compose the reproductive system, and in the same glands and tissues of different animals. The concentration of zinc in the *testis* was low in most cases, Bertrand reporting from 14 to 21 mg. of zinc per kilogram with one exception[8] for the gray rat, sheep, pig, bull, and man, though in the testicle of the rabbit his findings were 38 to 76 mg. of zinc per kilogram, and in the horse, 214 to 227 mg. Because of the lack of consistency, it is useless to speculate on these results, and it seems apparent

TABLE 1.—THE NORMAL CONCENTRATION OF ZINC IN THE TISSUES OF

ANIMAL	CONCENTRATION OF ZINC IN									
	Blood		Liver		Kidney		Spleen		Lung	
	mg.	*ref.*[1]	*mg.*	*ref.*	*mg.*	*ref.*	*mg.*	*ref.*	*mg.*	*ref.*
Man	20.6	(24)	10.0– 76.0	(33)	68.0	(40)	42.0	(40)	6.1– 7.1	L
	4.8– 5.8	L	39.0–145.0	(36)	19.5–49.0	L	10.0–12.7	L		
			52.0–145.5	(6)						
			115.0	(40)						
			33.6– 68.3	L						
Cattle	5.3– 5.7	(34)	58.0– 84.0	(34)	24.0	(31)	13.5	(6)		
	21.4	(24)	35.5– 83.3	(6)						
	5.1	(14)	42.0	(31)					....	..
			43.0	(24)						
Dog	18.4–22.5	(24)	42.5	(6)	30.0	(24)	23.0–29.0	(24)	10.3	(6)
	4.0–5.0	...[2]	36.0	(24)						
Horse	15.1	(24)	339.0	(6)	57.0	(41)	45.0	(41)	39.0	(41)
			75.0	(41)					11.0	(6)
Rabbit	19.0–26.1	(24)	19.3	(6)	43.0	(24)			36.0	(31)
	38.0	(31)	30.0–135.0	(31)	32.0	(31)	....	..		
			45.0– 48.0	(24)						
Cat	4.2– 5.1	L	27.1– 61.7	L	12.2–15.7	L	7.1–18.8	L	12.4–16.2	L
Rat	5.4– 7.5	L	18.2– 23.8	L	11.0–19.0	L	27.7–43.1	L	15.6–33.3	L

[1] Figures in parentheses refer to authors listed in the bibliography. "L" refers to results obtained

[2] Fairhall, unpublished data.

[8] Two analyses made on the testicle of man gave 16 and 76 mg. of zinc per kilogram.

that very considerable work will be necessary, including many carefully controlled analyses, before even a mere

unusually high concentration of the metal. These generalizations have, however, been borne out only by

VARIOUS ANIMALS: SUMMARY OF RESULTS REPORTED IN THE LITERATURE

MILLIGRAMS PER KILOGRAM

Brain		Testicle		Heart		Muscle		Total Animal	
mg.	*ref.*	*mg.*	*ref.*	*mg.*	*ref.*	*mg.*	*ref.*	*mg.*	*ref.*
5.2-15.7	(42)	16.0- 76.0	(27)	12.6-15.6	L	25.6-36.0	L		
8.3	L	8.0- 10.3	L			47.1-51.5	(6)	....	...
11.0	(36)								
10.6-12.5	(6)								
9.0	(6)	18.0	(27)			21.0	(24)		
17.0	(31)					26.3	(12)		
(calf)				....	...	25.0-37.0	(31)	....	...
						46.0-52.0	(34)		
						26.0-50.0	(36)		
						46.8-50.4	(6)		
87.0-92.0	(24)	....	...	4.0	(6)	20.0	(24)	....	...
6.1	(6)					43.1	(6)		
43.0-62.0	(41)	214.0-227.0	(41)	15.8	(6)	29.0	(6)	....	...
				71.0	(41)	55.0-60.0	(41)		
31.0-64.0	(31)	38.0- 76.0	(31)	45.0	(31)	20.0	(24)	31.0-49.0	(28)
						17.0-23.0	(31)	12.0-45.0	(43)
9.0-10.0	L	5.7- 11.8	L	....	...	21.0-21.3	L	31.1-36.5	L
10.4-20.7	L	8.5- 23.1	L			7.2-17.3	L	25.0-42.0	(28)
		21.0 (gray rat)	(27)	....	...			(mice) 22.2-34.5	L

by myself and given in the experimental part of this paper.

empirical and analytic answer can be given to the problem undertaken by these men.

Certain investigators have indicated various organs, particularly the reproductive organs and the thymus, as containing the largest amounts of zinc. It has been claimed by Giaya (32) and Delezenne (24) that the *brain* and *nervous tissue* also possess an

the results of the authors who made them, and are not confirmed by the analyses of other investigators, including Bodansky (42) and Rost and Weitzel (6) (36). The results of these three workers on the zinc content of human brain are in accord, and indicate, contrary to those of Delezenne and Giaya, a low and fairly constant concentration of the metal, from 5.2 to

15.7 mg. per kilogram. Rost and Weitzel found 6.1 mg. of zinc per kilogram in the brain of the dog, as against 87 to 92 mg., as reported by Delezenne. Bertrand (31) (41) reported concentrations of zinc in the brain of the rabbit and the horse from 31 to 64 mg. per kilogram, results which are high and contradictory to the figures of Rost and Weitzel, if one assumes that brain is, in general, similarly constituted in different animals. Bertrand, however, obtained lower results in his analyses of the brain of the calf and the sheep (17 and 18 mg. per kilogram, respectively). Rost and Weitzel obtained 6 mg. per kilogram in the brain of the pig and 9 mg. per kilogram in the brain of cattle. It is apparent, then, that zinc is an important mineral constituent of the brain, and that the concentration is small, fairly constant—if one disregards the results of Bertrand and Delezenne—and of the same magnitude in different animals.

The concentration of zinc in the *voluntary muscle* of man, cow, dog, horse, and rabbit, ranges from 17 to 60 mg. per kilogram according to a number of relatively uniform determinations, and these results (Table 1) indicate that the zinc concentration in the voluntary muscle of any animal is under normal conditions approximately constant. For the *heart* of the horse, Rost and Weitzel reported 15.8 mg. per kilogram, whereas Bertrand found 71 mg. For normal human *uterus*, Cristol (40) reported 32 mg. of zinc per kilogram.

For bone and hair, there have been reported but few analyses. Several investigators, including Delezenne, have reported positive results in testing for zinc in *bone*, but the only quantitative result reported is that of Bertrand (41), who found a large amount of the metal in the bone of the horse (310 mg. per kilogram) and only a small concentration (32 mg. per kilogram) in the marrow. In general, investigators have considered that zinc is absent from bone, or present only in traces. Such a conclusion is, however, not in accord with my findings, which indicate that the concentration of zinc in bone is very high.

In human *hair*, Rost found 9 mg. per kilogram but Bertrand reported 65 mg. per kilogram in the hair of rabbits and the same concentration in the hide. Delezenne found in the hair of the horse over 200 mg. per kilogram (254 mg. per kilogram of dry material), and approximately 150 mg. per kilogram for *hoof* (157 mg. per kilogram of dry material). Zinc was also found in unusually large amounts in human skin. My results on human and cat hair indicate that the concentration of zinc in hair is remarkably high. In this connection, however, a cautious investigator should always consider the greater possibility of the contamination of hair and skin with zinc than of any of the internal organs or tissues.

In a series of experiments on different animals, Bertrand and Vladesco (43) (28) followed the *total zinc concentration* and attempted to show a relation between the age and concentration of zinc. According to their analyses, rabbits varied in zinc concentration from 12 to 49 mg. per kilogram, a maximum being present at birth, the concentration declining during the period of lactation, and

rising rapidly again following weaning. The total zinc concentration in rabbits one to four months old was from 44 to 49 mg. per kilogram, in mice from 25 to 42 mg., and in the guinea-pig from 23 to 56 mg. (two observations). These results suggest the interesting possibility that the total zinc concentration varies but little in animals of different species. My findings of 29.4 mg. of zinc per kilogram for the rat and of 33.2 mg. per kilogram for the cat offer confirmatory evidence on this point.

A great deal of interest has been shown in the mineral constituents of *tumors*, and the amounts of zinc present, determined by Cristol (40), have been found to vary with the type of tumor. In benign conjunctival tumors, according to this author, zinc is slightly increased above the normal; in epithelial tumors, the increase is more marked. The zinc content depends on the nature of the tumor; fibrous tumors contain less than epithelial tumors; the greater the amount of connective tissue, the less the zinc. The amount of zinc is parallel to the degree of karyokinesis; the more rapid the evolution of the tumor, the greater the zinc content. Taverne (44), however, observed little difference in the zinc content between normal mammary gland (3.74 mg. per kilogram) and the cancerous part of a mammary carcinoma (3.65 mg. per kilogram).

Summary.—The normal and universal occurrence of zinc has been clearly shown in plants of all types, in a wide variety of foods derived from plants, and in all the tissues and organs of marine organisms, molluscs, reptiles, fish, fowl, and mammals. In few, if any, natural biologic materials which have been analyzed, has there been reported failure to detect zinc. We may, therefore, consider as established the fact that zinc is a universal and normal mineral constituent of all biologic material.

The concentrations of zinc reported were, in many cases, markedly contradictory. Obviously, therefore, the hypothesis that the normal concentration of zinc in any given animal or plant tissue is approximately constant has not been established, but because of the doubtful accuracy and general inadequacy of the data available, the hypothesis is by no means disproved. The experiments to be reported in the following section of this paper afford considerable positive evidence in affirmation of this hypothesis, a discussion of which will be reserved until then.

III. A Study of the Normal Distribution of Zinc in the Rat, Cat, and Man

A. Analytic Methods

The investigation of the distribution of zinc in the rat, cat, and man has involved a large number of analyses of biologic materials which varied in nature, in composition, and in quantity. The divergencies among many determinations of normal zinc concentrations as reported in the literature are probably due to inadequate attention to the analytic method, and I consider it of importance, therefore, to give a brief account of this phase of the experimental work.

In order to obtain data on the zinc content of the organs of the rat where small amounts of material of the order of 1 gm. were analyzed, the new

method which I developed and discussed in a previous paper (14) was used—a method based on the fluorescence produced by the action of zinc salts on urobilin. In all of the experimental work, the procedure as described in my early paper was followed and a final solution of zinc was obtained which was free from the impurities present in the original sample. The procedure involved the following steps: ashing of material at 450° to 550°C., extraction with hydrochloric acid, precipitation of iron as phosphate by means of sodium acetate and reprecipitation in the presence of large amounts of iron, addition of an entrainer to carry down the precipitate of zinc sulphide (copper), precipitation of copper and zinc as sulphides, reprecipitation in certain cases to complete the separation, precipitation of the sulphides of the copper group metals from an acid solution, and evaporation of excess acids to obtain the pure zinc salt as a final residue. Careful technic was found to be essential when the method was used in the determination of amounts of zinc as low as 0.01 mg., but satisfactory results were obtained when every precaution was taken.

The ferrocyanide turbidimetric method (45) (46), formerly used for analysis of amounts of zinc ranging from 0.5 to 5 mg., was used in a few cases in this research, but was discarded because it was found not only that the urobilin method was more sensitive, but also that it was sufficiently accurate[9] and was more convenient with regard to laboratory manipulation. The urobilin method was, therefore, used for practically all of the experiments on samples containing amounts of zinc under 1 to 2 mg.

It is perhaps desirable to indicate and to emphasize certain necessary precautions. The isolation of the zinc as outlined must be made in every case, although with large amounts of zinc (above 1 to 2 mg.), where the volumetric method is used, extreme care is found necessary only in those steps where loss of zinc is possible.[10] The urobilin test, however, is affected as much by impurities remaining in the final sample of zinc as it is by the actual loss of zinc in the procedure; it is necessary, therefore, to take the utmost care in manipulation and technic. If small amounts of sodium, calcium, or magnesium appear in the final solution of zinc, the test with urobilin is often seriously impaired. The important part of the procedure, then, is the treatment of the precipitate of the zinc sulphide entrained with the sulphides of the metals of the copper group. Careful washing of this precipitate is essential, especially when any considerable amounts of other salts—even sodium salts—are present, and it is often found advisable to carry out a reprecipitation of the zinc sul-

[9] According to the literature, the turbidimetric methods are capable of slightly greater accuracy under ideal conditions than has been claimed for the urobilin method. It is the author's belief, however, that there is little, if any, difference in the accuracy attainable in the two methods under the conditions of these experiments. It should be stated at this point (in correction of an error (14, p. 287)) that the sensitivity of the turbidimetric method is approximately 0.03 to 0.05 mg. in 50 c.c., whereas the urobilin method is capable of detecting 0.001 mg. in the same volume.

[10] Thus, reprecipitation of the sulphides to remove traces of included salts was never necessary, but the reprecipitation of large amounts of ferric phosphate was important.

phide in order to insure complete removal of the soluble metallic salts. Following this point in the procedure, only acids are added and, after the precipitation of the sulphides of the copper group metals, these are completely expelled by evaporation.

In a few analyses reported in this paper, iron was removed by precipitation in strong acid solution with cupferron, but the use of this reagent was not developed until the latter part of the investigation, and its advantages have not yet been definitely established. It gave excellent service in connection with the volumetric analysis of large amounts of zinc, and avoided the time consuming filtration of ferric phosphate, but it involved a disadvantage, in that organic material occasionally remained in the solution and interfered to some extent in the procedure employed with small amounts of zinc.

A procedure was adapted for the determination of amounts of zinc above 1 to 2 mg., from the well-known standard methods (47) used in the volumetric determination of relatively large amounts of the metal. A brief description of this procedure is as follows: An ammoniacal solution of zinc containing iron held in solution as the citrate is titrated with potassium ferrocyanide, the zinc being precipitated as zinc ferrocyanide; and the end point is found by testing a drop of the mixture with 50 per cent. acetic acid on a spot plate, an immediate precipitate of Prussian blue showing the presence of an excess of potassium ferrocyanide. The method has ordinarily been used to determine relatively large amounts of zinc. In this investigation it has been applied on a small scale and has been found satisfactory for the analysis of zinc in amounts as low as 1 to 2 mg. In carrying out an analysis, the ordinary procedure is followed, one-tenth of the quantities of materials being used with correspondingly small concentrations. In a typical case, for the analysis of a sample containing approximately 3 mg. of zinc, the volume of solution of the zinc, isolated according to the usual procedure, is made 10 c.c. and is titrated from a burette, graduated in twentieths of a cubic centimeter, with a standard solution of potassium ferrocyanide of such a strength as to precipitate 1 mg. of zinc per cubic centimeter. Thus, the total volumes of solution are small, as are the concentrations. The zinc solution, treated with a standard volume of ferric citrate solution and ammonia until it is alkaline, is heated to boiling and titrated while hot. The standard solution of ferrocyanide should always be tested before each series of analyses to eliminate any possible error due to a change in strength on standing.

The method requires, furthermore, the determination of standardization factors for the ferrocyanide solutions for the varying amounts of zinc in the samples analyzed, because the end point is affected by concentrations, which must vary if one starts with standard initial volumes, ferrocyanide solution, and total ferric citrate added. A number of trial titrations of known amounts of zinc should be made before each series of analyses and factors for the ferrocyanide solution obtained for varying amounts of zinc under standard initial conditions. The factor for the ferrocyanide solution should be chosen to correspond with the con-

centration of zinc in the unknown sample, as determined roughly from the number of cubic centimeters of standard ferrocyanide solution used up in the titration. Although no one standard set of conditions was observed in the work reported in this paper, the experiments were carefully controlled in this general manner. The results obtained by this method were often checked by first analyzing the sample by the urobilin method. Since only a small fraction of the sample was required for this procedure, the remainder was titrated and corrected for the portion removed.

B. Distribution of Zinc in the Rat

The rats (*Mus norwegicus albinus*) used as subjects in these experiments were kept in copper cages on a uniform diet for a period of at least ten days before analysis in order to prevent any possible contamination with zinc from galvanized iron cages. The diet of the rats consisted of a mixture of rolled oats (5 parts), hominy (3 parts), milk powder (Klim, $1\frac{1}{2}$ parts), beef scraps ($2\frac{1}{2}$ parts), salt (traces), with a supplementary ration of molasses horse feed, dog biscuit, and lettuce or cabbage. Table 2 contains the results of zinc analyses of typical samples of these foods. On this diet, the average daily ingestion by an adult rat was approximately 0.77 mg. of zinc. The amount of zinc excreted in the feces per day was approximately equivalent—that is to say, the daily intake and output of zinc were approximately in equilibrium.

The procedure in any series of analyses was as follows: The animal was bled to death under chloroform anesthesia, and was then dissected with carefully cleaned instruments. The sample of blood thus obtained does not, of course, represent the total blood of the animal, but in most cases it does so very nearly; in any event, no error of serious theoretical consequence has been introduced. The hide (skin and hair) was completely removed. All materials were placed in porcelain dishes appropriate for ashing, and were weighed as soon as possible in order to minimize the loss of weight by evaporation of water. The viscera, with the exception of the heart and a few glands too small to be suitable for analysis, were removed, dissected free from adhering tissues, weighed, and set aside for analysis. The gastro-intestinal tract, exclusive of the esophagus, was slit open and was washed thoroughly with running water to remove the contents. The brain and spinal cord were removed and were analyzed together. The total residue of bone; claws; muscle; heart, esophagus, bladder, penis, blood vessels (all largely composed of muscle); trachea; omentum, peritoneum, connective tissue and fat; and a few glands (adrenals, pancreas, thymus, salivary glands) too tiny to be analyzed separately were boiled with water, the bone picked out for separate analysis, and the water extract and residue of muscle tissue, etc., dried and ashed. The bone was weighed and subtracted from the weight of the total residue to give the weight of muscle and miscellaneous tissue. The term "muscle" used in Tables 3 and 6 is, therefore, strictly speaking, not accurate since the analyses listed under it include all rat tissues not analyzed separately. The great bulk of the tis-

sue analyzed was, of course, muscle, and any changes in zinc concentration following zinc dosage would undoubtedly not affect this normal so-called muscle figure unless the great bulk of tissue included by it, the actual muscle, were involved. A single analysis made in this laboratory by Miss Marian Marsh on a 5-gm. sample of rat muscle dissected as clean as possible from other tissue showed a zinc concentration of 0.0155 mg. per gram, a figure very close to the average of 0.0136 mg. per gram given for muscle (muscle and miscellaneous tissue included) in Table 3.

The total male reproductive organs, exclusive of the penis (testes, vasa deferentia, seminal vesicles and contents, and accessory glands), were analyzed in a few cases, but usually only the testes were taken. With the exception of one female, all rats analyzed were males. In the one female rat, the ovaries, uterus, and tubes were analyzed together and a figure obtained for the total female reproductive tract. This figure has, however, not much significance since it represents the zinc content of both glandular and muscular tissue, the ovaries constituting only about one-fifth of the weight of the total female reproductive tract of the rat analyzed.

The total zinc content of the rat was determined by the summation of the analyses of the various tissues, and the total weight of the animal was deter-

TABLE 2.—ZINC CONTENT OF TYPICAL SAMPLES OF RAT FOOD

FOOD	WEIGHT OF SPECIMEN	ZINC FOUND	AVERAGE CONCENTRATION
	gm.	*mg.*	*mg. per kg.*
Mixture, rolled oats, hominy, powdered milk, beef scraps (2 mixtures sampled)	#1 10	0.42	42.00
	#1 10	0.42	
	#2 10	0.25	28.33
	#2 10	0.30	
	#2 10	0.30	
Molasses horse feed mixture	10	0.17	18.50
	10	0.20	
Lettuce	9	0.012	1.30
	9	0.012	
Cabbage	20	0.038	1.98
	20	0.038	
	20	0.043	
Dog biscuit[1]	100	3.40	34.00
	100	3.40	34.00

[1] Analyses of dog biscuit were made by Dr. P. K. Thompson.

mined by summing the weights of the organs and tissues after dissection, a figure being obtained which was usually 5 to 8 per cent. lower than the normal weight of the animal before dissection. This loss of weight was due mainly to the removal and discard of the intestinal contents, and was enhanced only to a slight extent by the evaporation of water from tissues before the determination of their weights, the latter factor being negligible as care was taken to weigh the organs as rapidly as possible after dissection.

In Table 3 have been listed the results of a series of analyses of ten rats, the weights of the tissues, the total zinc found, the concentration, and the average concentration. In this table, then, may be found all of the analytic data on rats of different ages and weights, and the range as well as the average of zinc concentration obtained.

A comparison of the relative concentrations of zinc in the various tissues, illustrated in Figure 1, reveals the striking fact that bone contains by far the highest concentration of zinc, from five to ten times that in any of the other tissues which were analyzed. The concentration of zinc in blood was the lowest. It is an obvious fact that in no organ or tissue is zinc absent or present in infinitesimal traces. Zinc is present in relatively large amounts in all tissues.

The analyses for zinc in blood were approximately constant, varying from 5.4 to 7.5 mg. per kilogram in nine individuals (6.1 to 7.5 mg. per kilogram in eight cases), and were much lower than the figures given by Bertrand and Delezenne for the blood of many other animals. The conclusion may be drawn that zinc concentrations in blood of the rat are normally constant.

The same constancy of results has

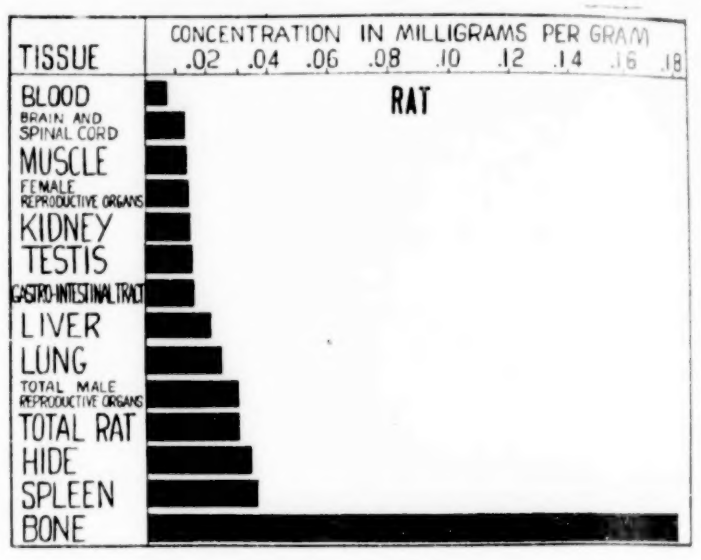

FIG. 1.—The normal concentration of zinc in the tissues and organs of the rat.

been obtained in many other organs; in the gastro-intestinal tract the concentration of zinc ranged from 10.8 to 21.7 mg. per kilogram in nine cases; in the liver, from 18.2 to 23.8 in nine cases; in the kidneys, 11 to 19 in seven cases; in the spleen, 27.7 to 43.1 in six cases; in the total reproductive organs, from 27 to 32.3 in five cases; in brain, from 10.4 to 20.7 in five cases; in muscle, from 10.6 to 17.3 in eight cases (7.2 and 8.3 in two cases); in hide, from 21.5 to 41.3 in ten cases; in bone, from 152.9 to 216.7 in nine cases (125 in the tenth case); and in the total rat, from 22.2 to 34.5 mg. in ten cases. In the few organs and tissues not enumerated there were some greater variations in zinc concentration, but these were not of such magnitude as to preclude the conclusion that the concentrations of zinc in the various organs and tis-

TABLE 3.—NORMAL DISTRIBUTION OF ZINC IN THE RAT

TISSUE	RAT NO.	WEIGHT OF TISSUE	TOTAL ZINC FOUND	CONCENTRATION OF ZINC	AVERAGE CONCENTRATION OF ZINC
		gm.	*mg.*	*mg. per gm.*	*mg. per gm.*
Blood	2	1.8	0.012	0.0067	0.0067
	3	3.5	.019	.0054	
	4	4.4	.027	.0061	
	5	3.6	.027	.0075	
	6	6.4	.046	.0072	
	7	6.1	.038	.0062	
	8	7.5	.056	.0074	
	9	5.1	.033	.0065	
	10	7.8	.058	.0074	
Gastro-intestinal tract	1	4.0	0.048	0.0120	0.0151
	2	6.0	.076	.0127	
	3	5.6	.085	.0152	
	5	9.2	.200	.0217	
	6	7.7	.110	.0143	
	7	9.0	.100	.0111	
	8	9.0	.170	.0189	
	9	9.3	.100	.0108	
	10	8.8	.170	.0193	
Liver	1	3.3	0.060	0.0182	0.0207
	2	3.7	.088	.0238	
	4	5.8	.119	.0205	
	5	8.6	.180	.0209	
	6	9.1	.195	.0214	
	7	7.8	.160	.0205	
	8	10.6	.200	.0189	
	9	10.4	.220	.0211	
	10	11.1	.230	.0207	
Kidneys	1	0.45	0.0050	0.0111	0.0144
	3	0.85	.0145	.0171	
	4	1.00	.0110	.0110	
	5	1.50	.0240	.0160	
	6	1.70	.0190	.0112	
	7	1.80	.0280	.0156	
	10	2.00	.0380	.0190	
Spleen	1	0.09	0.0035	0.0389	0.0363
	6	.90	.0290	.0322	
	7	.50	.0180	.0360	
	8	.45	.0180	.0400	
	9	.65	.0180	.0277	
	10	.65	.0280	.0431	

TABLE 3.—*Continued*

TISSUE	RAT NO.	WEIGHT OF TISSUE	TOTAL ZINC FOUND	CONCENTRATION OF ZINC	AVERAGE CONCENTRATION OF ZINC
		gm.	*mg.*	*mg. per gm.*	*mg. per gm.*
Lungs	2	0.50	0.015	0.0300	0.0236
	3	0.60	.020	.0333	
	4	0.95	.022	.0232	
	5	1.15	.025	.0217	
	6	1.50	.036	.0240	
	7	1.40	.028	.0200	
	8	1.30	.028	.0215	
	9	1.35	.031	.0230	
	10	1.60	.025	.0156	
Testes	1	0.47	0.004	0.0085	0.0149
	2	0.65	.015	.0231	
	4	1.90	.025	.0132	
Total male reproductive tract	5	4.30	0.130	0.0302	0.0293
	7	5.45	.160	.0294	
	8	6.20	.200	.0323	
	9	4.75	.130	.0274	
	10	7.40	.200	.0270	
Total female reproductive tract	3	0.35	0.005	0.0143	0.0143
Brain and spinal cord	1	1.30	0.015	0.0115	0.0134
	2	1.45	.030	.0207	
	3	1.85	.021	.0114	
	6	2.30	.024	.0104	
	9	2.30	.030	.0130	
Muscle	1	21.0	0.24	0.0114	0.0136
	2	25.5	0.40	.0157	
	3	52.0	0.55	.0106	
	4	57.0	0.41	.0072	
	5	119.0	2.00	.0168	
	6	118.0	1.80	.0153	
	7	127.0	2.20	.0173	
	8	124.0	2.10	.0169	
	9	151.0	1.25	.0083	
	10	144.0	2.35	.0163	
Hide (hair and skin)	1	6.0	0.21	0.0350	0.0338
	2	10.4	0.30	.0288	
	3	20.5	0.44	.0215	
	4	24.0	0.73	.0304	
	5	45.5	1.88	.0413	
	6	46.0	1.25	.0272	
	7	50.0	2.00	.0400	
	8	56.0	2.20	.0393	
	9	57.0	2.13	.0374	
	10	58.0	2.13	.0367	

TABLE 3.—*Concluded*

TISSUE	RAT NO.	WEIGHT OF TISSUE	TOTAL ZINC FOUND	CONCENTRATION OF ZINC	AVERAGE CONCENTRATION OF ZINC
		gm.	*mg.*	*mg. per gm.*	*mg. per gm.*
Bone	1	3.0	0.63	0.2100	0.1784
	2	4.0	0.81	.2025	
	3	7.5	1.32	.1760	
	4	8.0	1.00	.1250	
	5	12.0	2.60	.2167	
	6	14.0	2.14	.1529	
	7	13.0	2.70	.2077	
	8	14.0	2.50	.1786	
	9	17.0	2.75	.1618	
	10	17.0	2.60	.1529	
Total rat	1	41.0	1.23[1]	0.0300	0.0294
	2	55.0	1.77	.0322	
	3	98.0	2.59	.0264	
	4	113.0	2.51	.0222	
	5	206.0	7.10	.0345	
	6	211.0	5.74	.0272	
	7	224.0	7.46	.0333	
	8	233.0	7.53	.0323	
	9	261.0	6.72	.0257	
	10	261.0	7.86	.0301	

[1] The values for the total zinc content of the rats have been obtained by summation of the amounts of zinc found in the various organs and tissues. In the cases where the analyses of certain of the organs were lost an approximate figure has been used, calculated from the weight of the organ (not included in this table) and the average concentration of zinc in the organ as determined. Inasmuch as the weights of the organs lost were small, and the calculated values reasonably accurate, no appreciable error has been introduced.

sues of the rat may be considered as normally constant within a small range. The figures for the total rat concentrations are especially interesting, in showing approximately equal concentrations for ten rats varying in weight, through an ascending series, from 41 gm. to 261 gm. Thus, we have equal concentrations of zinc in a recently weaned animal three to four weeks old, through rats of increasing age and size, up to an adult animal, approximately twenty-three weeks old.

It may be suggested that, if the figures for concentrations were based on the dry weights of the tissues, the differences might disappear, inasmuch as bone is relatively dehydrated as compared to other tissues. Such a correction,[11] while tending to equalize concentrations, does not eliminate the differences. Bone still contains, per gram of dehydrated material, approximately twice as much zinc as any other tissue.

The mouse contains approximately

[11] In all the dry weight calculations made, the percentage of water was estimated approximately (35) (48) (49) from the known water content of similar tissues in other animals.

100 mg. of iron per kilogram (35). Assuming the same for the rat, it is interesting to note that the zinc concentration in the total animal is 29.4 mg. per kilogram, a quantity nearly one-third of the concentration of iron. Contrary to iron, however, zinc is fairly evenly distributed throughout the tissues. In bone there is only an infinitesimal trace of iron, whereas zinc is present in very considerable amounts.

The maximum amount of zinc localized in any one tissue in the body

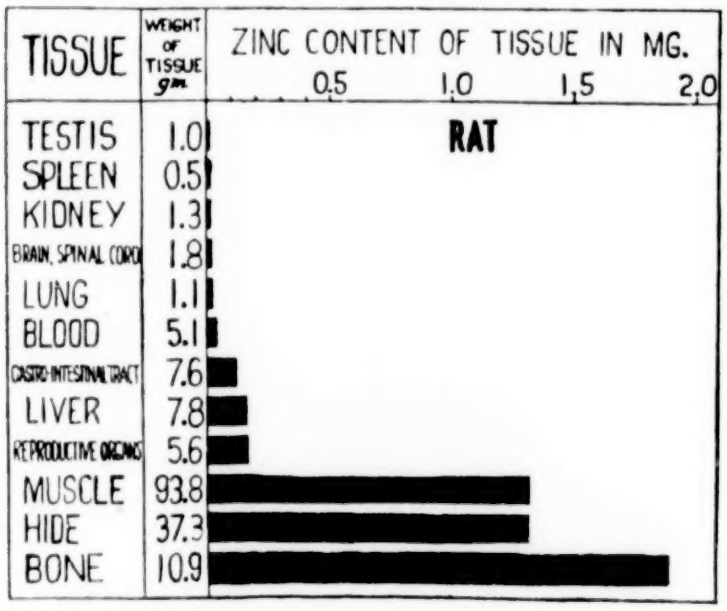

FIG. 2.—The normal localization of zinc in the tissues and organs of the rat.

exists in bone but, as shown in Figure 2, zinc also occurs in considerable amounts in muscle and hide. The total amounts of zinc localized in the other organs and tissues are relatively small. The analysis of the total male reproductive organs indicates a rather high concentration of zinc and a localization approximately equal to that of liver. This fact corroborates, to a certain extent, an observation made by Bertrand (27) that the concentration of zinc in certain of the reproductive glands was relatively high, though low in the testis.

C. Distribution of Zinc in the Cat

The cats studied were kept on an approximately constant diet (milk and salmon), were bled to death under chloroform anesthesia, and were dissected and analyzed in a manner exactly similar to that described for the rat. The analytic results are listed in Table 4. The term "muscle" has again been applied to the total muscle mass of the animal plus the organs and tissues which were not isolated and analyzed separately. Several organs of the rat which were too small to analyze were studied in the case of the cat. These included the gallbladder and its contents, the thyroid, the submaxillary gland, the adrenals, and the pancreas. It is to be noted that the quantities of materials obtained for analysis were much greater for the cat than for the rat, and the results obtained, therefore, were less liable to occasional analytic error. For this reason, it was not necessary to carry out so large a number of distribution determinations.

The analysis of the blood ranged from 4.2 to 5.1 mg. of zinc per kilogram in four individuals, and in many other tissues there was found a similarly constant series of concentrations of zinc. The ranges in milligrams per kilogram were as follows:

	Mg. per Kg.	*No. of Cases*
Gastro-intestinal tract	16.8 to 21.7	3
Pancreas	23.1 to 27.7	3
Gallbladder and contents	4.0 to 8.0	3
Kidneys	12.2 to 15.7	4
Lungs	12.4 to 16.2	3
Testes	5.7 to 11.8	3
Brain and spinal cord	9.0 to 10.0	3

	Mg. per Kg.	*No. of Cases*
Muscle.....	21.0 to 21.3	2
Hide.....	30.6 to 54.6	3
Hair.....	208.7 to 241.4	3
Bone.....	120.1 to 133.3	3
Total cat.....	31.2 to 36.4	3

The zinc concentrations in spleen were not very constant (7.1 to 18.8 mg. per kilogram in five cases). In the liver, also, there appeared considerable variation, from 27.1 to 61.7 mg. per kilogram in six individuals, but except for one high and one low result, there was a remarkably close agreement of 35 to 43.8 mg. per kilogram for four cases. Because of the nature and function of the liver, this variation in concentration will not invalidate the obvious conclusion to be drawn from the mass of the results. The concentration of zinc in any given tissue of the cat, under normal and standard conditions, is constant within a small range.

There is a general gradation of the average concentrations of zinc in all tissues of the cat, as is shown in Figure 3, from a low point in blood, approximately 5 mg. per kilogram, to the very high figure of 224 mg. per kilogram in hair. Contrary to the results obtained with the rat, the spleen was relatively low in zinc and the liver richer in this metal. Of the viscera, the liver, thyroid, and adrenals showed the highest zinc concentration.

The outstanding fact that large amounts of zinc occur in bone is overshadowed by the tremendous amounts of the metal found in the hair. The concentration of zinc in bone is three times that of the liver but slightly more than one-half that found in hair. This extremely high concentration of zinc in hair is relatively constant (from 208.7 to 241.4 mg. per kilogram

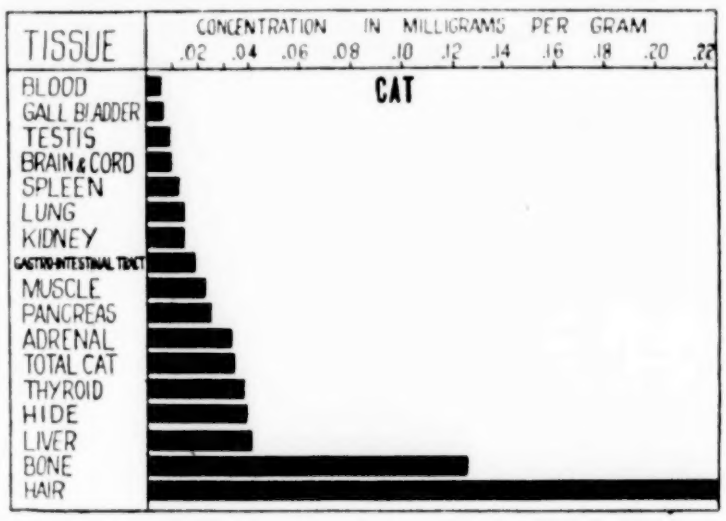

FIG. 3.—The normal concentration of zinc in the tissues and organs of the cat.

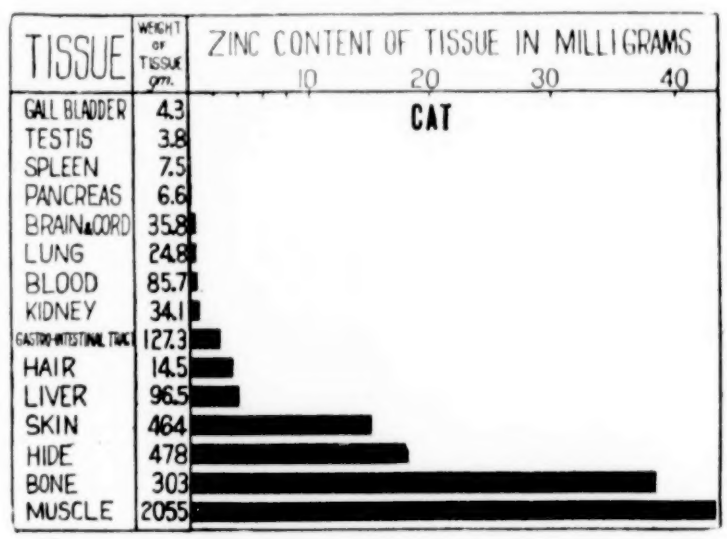

FIG. 4.—The normal localization of zinc in the tissues and organs of the cat.

in three cases), a fact which accounts for the relatively high zinc content of the hide, not only of the cat but also of the rat. The greater possibility of the contamination of hair and skin than of any of the internal organs or tissues with zinc from some outside source should, however, again be mentioned, though the uniformity of our results seems to be answer to this objection.

A calculation of the concentration of zinc in the tissue, in terms of dry

TABLE 4.—NORMAL DISTRIBUTION OF ZINC IN THE CAT

TISSUE	CAT NO.	WEIGHT OF TISSUE	TOTAL ZINC FOUND	CONCENTRATION OF ZINC	AVERAGE CONCENTRATION OF ZINC
		gm.	*mg.*	*mg. per gm.*	*mg. per gm.*
Blood	1	53.0	0.27	0.0051	0.0046
	2	110.0	.51	.0046	
	3	45.0	.20	.0044	
	4	135.0	.57	.0042	
Gastro-intestinal tract	1	120.0	2.6	0.0217	0.0191
	2	155.0	2.9	.0187	
	3	107.0	1.8	.0168	
Pancreas	1	6.5	0.150	0.0231	0.0248
	2	6.5	.180	.0277	
	3	7.0	.165	.0236	
Liver	1	81.0	5.00	0.0617	0.0411
	2	118.0	3.20	.0271	
	3	100.0	3.50	.0350	
	4	96.0	4.20	.0438	
	5	101.0	4.15	.0411	
	6	83.0	3.12	.0376	
Gallbladder and contents	1	5.0	0.040	0.0080	0.0057
	2	5.0	.020	.0040	
	4	3.0	.015	.0050	
Kidneys	1	41.0	0.56	0.0137	0.0141
	2	42.5	.62	.0146	
	3	30.0	.47	.0157	
	4	23.0	.28	.0122	
Spleen	1	8.5	0.060	0.0071	0.0126
	2	7.0	.072	.0103	
	3	6.0	.113	.0188	
	4	7.5	.098	.0131	
	5	8.9	.120	.0135	
Lungs	1	34.0	0.42	0.0124	0.0145
	2	18.5	.30	.0162	
	3	22.0	.33	.0150	
Testes	1	4.5	0.053	0.0118	0.0085
	2	3.5	.028	.0080	
	3	3.5	.020	.0057	
Adrenals (4 animals)		1.97	0.065	0.0330	0.0330
Submaxillary		2.20	0.022	0.0100	0.0100
Thyroids (4 animals)		1 03	0.039	0.0379	0.0379
Brain and spinal cord	1	37.00	0.34	0.0092	0.0094
	2	35.50	.32	.0090	
	3	35.00	.35	.0100	

TABLE 4.—*Continued*

TISSUE	CAT NO.	WEIGHT OF TISSUE	TOTAL ZINC FOUND	CONCENTRATION OF ZINC	AVERAGE CONCENTRATION OF ZINC
		gm.	*mg.*	*mg. per gm.*	*mg. per gm.*
Muscle	1	1970.00	42.00	0.0213	0.0211
	2	2040.00	...[1]	...[1]	
	3	2140.00	45.00	.0210	
Hide (total)	1	366.00	20.00	0.0546[2]	0.0391
	2	499.00	16.00	.0321	
	3	571.00	17.50	.0306	
Hair[3]	1	18.00	4.00	0.2222	0.2241
	2	14.00	3.38	.2414	
	3	11.50	2.40	.2087	
Bone	1	333.00	40.00	0.1201	0.1259
	2	322.00	40.00	.1242	
	3	255.00	34.00	.1333	
Total cat	1	3.06 kg.	111.49	0.0364	0.0332
	2	3.36 kg.	(107.19)[1]	(.0314)[1]	
	3	3.32 kg.	103.45	.0312	

[1] The analysis for the muscle of Cat 2 was lost, and in order to calculate the total zinc content in the animal the concentration of zinc in muscle was assumed to be the average found in the two successful determinations.

[2] The hides of Cats 2 and 3 contained considerable adhering fat, while the hide of Cat 1, a very lean animal, contained practically none. Since the total zinc contents of the three hides, irrespective of weight, were approximately the same (the lean hide, in fact, containing slightly more zinc than the others), the conclusion seems highly probable that fat contains very little zinc, and when present in, or adhering to, a tissue to any considerable degree merely serves to reduce the figure for zinc concentration.

[3] Samples of hair were analyzed separately to determine the concentration of zinc, but the results were added to the analytic results for hide to obtain the figures given in this table for total hide.

weight, revealed but one striking change in relative zinc concentrations. The liver, on a dry weight basis, contained a concentration of zinc approximately equal to that of bone.

In Figure 4 is indicated the relative localization of zinc in the various organs and tissues of the cat. In the hide there is approximately 17 per cent. of zinc, while the bone and muscle each contain approximately 35 to 40 per cent. of the total zinc. The actual quantities in the other organs and tissues are relatively small.

D. Distribution of Zinc in Human Tissue

It was not possible, of course, to control the experiments on human tissues, but only those cases were studied, which, as far as could be determined from the medical histories, were perfectly normal with respect to contact with zinc. The organs and tissues, with the exception of the blood specimens, were obtained at autopsy as soon as possible after death. The blood samples were taken by venous

puncture from four different laboratory workers. The amounts of materials varied, and the determinations made are listed in Table 5 as observed concentrations and averages. Since it was not possible to obtain complete data on the total weights of the tissues, I have confined attention to concentration only.[12]

The agreement of the various analyses of different tissues and organs has been satisfactory in all except two cases. In blood, the zinc concentration varied from 4.8 to 5.8 mg. per kilogram in four cases; in stomach, from 9.5 to 12.6 in three cases; and in spleen, from 10 to 12.7 in three cases, etc. (see Table 5). Considerable variation was observed, however, in the kidneys and liver. In the kidneys, the zinc concentration ranged from approximately 20 to 49 mg. per kilogram in five cases, but in four of these the range was from 34 to 49 mg., which represents a reasonable agreement. In the liver, the concentration of zinc varied even more markedly—from approximately 34 to 68 mg. per kilogram in four cases. It is apparent that the liver in man, as an important excretory organ and with a function of storage of many materials, is subject to considerable variation in zinc content, depending on varying conditions and varying diet. In this connection, it should be noted that the rats and the cats analyzed had all had a preceding period of uniform diet. It is reasonable to expect that the analytic data on human liver would vary to some

[12] Making use of the average normal weights of the various tissues and organs, it would be a simple matter to calculate from the data assembled the approximate total zinc content of the organs and the approximate localization of zinc in man.

TABLE 5.—NORMAL DISTRIBUTION OF ZINC IN MAN

TISSUE	CONCENTRATION OF ZINC	AVERAGE CONCENTRATION OF ZINC
	mg. per gm.	*mg. per gm.*
Blood	0.0048	0.0052
	.0053	
	.0050	
	.0058	
Stomach	0.0108	0.0110
	.0126	
	.0095	
Small intestine	0.0086	0.0106
	.0092	
	.0140	
Large intestine	0.0152	0.0123
	.0119	
	.0097	
Liver	0.0336	0.0549
	.0683	
	.0545	
	.0630	
Kidney	0.0360	0.0350
	.0195	
	.0490	
	.0340	
	.0364	
Pancreas	0.0097	0.0124
	.0102	
	.0132	
	.0164	
Spleen	0.0113	0.0113
	.0100	
	.0127	
Lung	0.0071	0.0068
	.0071	
	.0061	
Testis	0.0080	0.0089
	.0103	
	.0085	

TABLE 5.—*Continued*

TISSUE	CONCENTRATION OF ZINC	AVERAGE CONCENTRATION OF ZINC
	mg. per gm.	*mg. per gm.*
Ovary	0.0139	0.0096
	.0053	
Adrenal	0.0049	0.0082
	.0083	
	.0089	
	.0106	
Brain	0.0083	0.0083
Muscle	0.0290	0.0302
	.0256	
	.0360	
Heart	0.0126	0.0141
	.0156	
Hair	0.1790	0.1630
	.1570	
	.1520	
Bone:		
Rib	0.0950	0.1008
	.0970	
	.1200	
Sphenoid	.1020	
Femur	.0900	

extent, in view of the fact that the cases studied were all pathologic. In no case was death sudden or the result of accident. In spite of this, our data indicate that practically all human organs and tissues, except the liver, contain zinc in concentrations which are very nearly constant in different individuals.

The average concentrations of zinc determined for human tissues and organs have been charted in Figure 5. It is evident from this figure that many of the organs in man show a relatively similar concentration, the variation in blood, lungs, brain, adrenals, pancreas, testes, ovaries, gastro-intestinal canal, spleen, and heart being only from 5.2

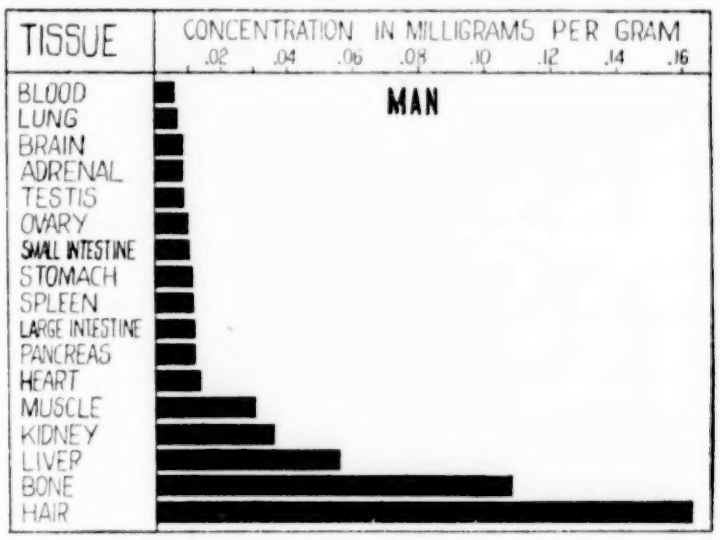

FIG. 5.—The normal concentration of zinc in the tissues and organs of man.

to 14.1 mg. per kilogram. Muscle, kidney, and liver are relatively high in zinc, while the concentration in bone is almost twice as great as in liver. Again, hair contains by far the highest concentration, a value very considerably above that reported by Rost. The total amount of zinc in man is extremely high. Calculating on the basis of the concentrations of zinc in the total cat and total rat, which are very close when reduced to an equivalent weight basis, the total zinc content of a man weighing 70 kg. is approximately 2.2 gm. This amount of zinc is very nearly as large as the probable total iron content which, calculated from Sherman's (50) percentage of iron in the body is approximately 2.8 gm. This high figure for total zinc in the human body is very suggestive of a probable biologic function of some importance.

Inspection of Table 1 shows that there are many discrepancies between

the values which I have determined and those reported in the literature. The analyses for blood indicate that the figures reported by Delezenne (24) are too high. For liver, a wide variety of results has been obtained, and my results, although not so extreme in either direction, are roughly in accord with those already reported. The figures reported for the concentration of zinc in human brain (Bodansky (42), Rost and Weitzel (6) (36)) are in excellent accord with those found by myself. Bertrand reported two widely divergent analyses for the testes, the lower of which was nearly twice as high as the average given in Table 5. For human muscle, the figures obtained by Rost and Weitzel show a range of 47.1 to 51.5 as contrasted with the values of 25.6 to 36 obtained in this research.

E. Comparison of the Zinc Concentrations in Rat, Cat, and Man

In Table 6 are included the average zinc concentrations of the various tissues in the three animal species studied, the rat, cat, and man. This comparison is of interest in order to determine to what extent the zinc content of a given tissue is constant in different animals. The agreement in the three species studied has been very close in the following tissues: blood, gastro-intestinal canal, brain, hide, and total animal. In certain other tissues—spleen, kidney, and testis—there is agreement in two species, while the third is somewhat, though not seriously, at variance. The concentration of zinc in the human kidney is more than twice as high as that of the rat and the cat, while the concentration in the testis, lung, and spleen of the rat is rather high as compared to the cat and man.

TABLE 6.—A COMPARISON OF THE NORMAL AVERAGE CONCENTRATIONS OF ZINC IN THE TISSUES AND ORGANS OF THE RAT, CAT, AND MAN

TISSUE	CONCENTRATION OF ZINC IN MILLIGRAMS PER GRAM		
	Rat	Cat	Man
Blood	0.0067	0.0046	0.0052
Gastro-intestinal tract	.0151	.0191	.0113
Pancreas	.....	.0248	.0124
Liver	.0207	.0411	.0549
Kidney	.0144	.0141	.0350
Spleen	.0363	.0126	.0113
Lung	.0236	.0145	.0068
Testis	.0149	.0085	.0089
Brain and spinal cord	.0134	.0094	.0083
Muscle	.0136	.0211	.0302
Hide	.0338	.0391	....
Hair	.....	.2241	.1630
Bone	.1784	.1259	.1008
Total animal	.0294	.0332	....

A real divergence appears in the liver and in muscle, where the zinc concentration is at a minimum in the rat, is approximately three times as high in man, and is approximately intermediate in the corresponding tissues of the cat. A reverse situation occurs with bone, where in the rat the zinc concentration attains a height nearly twice that in human bone. Again, in the cat, the concentration is intermediate. There is some variation between human and cat hair (not determined for the rat), that of the cat containing the higher concentration of zinc, although both figures are of the same order of magnitude. It is obvious, therefore, that one must be

cautious in assuming concentrations of zinc in a tissue of one species by analogy to that determined in some other animal, although such an approximation would in many cases probably not be far wrong.

F. Conclusion

A review of the literature has shown that the occurrence of zinc in plant and animal tissues in amounts above mere traces is normal and universal. This fact has been further confirmed by a complete study of animals of three different species, where, without exception, zinc was found present in every sample of every tissue analyzed.

The question as to whether or not the normal concentration of zinc in any particular biologic individual is constant was not conclusively answered by an examination of the data given in the literature. But the results of this study of three animal species, including, as they do, a large number of consistent and closely agreeing figures with no discrepancies of serious theoretical consequence, afford strong positive evidence on which to base the very probable hypothesis that in any given tissue of an animal species, under standard conditions, there is a definite normal concentration of zinc which is constant within a small range.

IV. Summary

In a review of the literature, the significance of the results reported on the normal occurrence of zinc in biologic materials has been discussed as showing that the occurrence is universal. The normal distribution of zinc has been determined in the tissues and organs of the rat, cat, and man. The results are shown to be consistent, and are made the basis of the hypothesis that the concentration of zinc in any species of tissue is normally constant within small limits.

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BOOKS RECEIVED

Books received are acknowledged in this column, and such acknowledgment must be regarded as a sufficient return for the courtesy of the sender. Selections will be made for review in the interest of our readers and as space permits.

LABOR RELATIONS IN INDUSTRY. By *Dwight Lowell Hoopingarner*, M.A., Executive Counsel on Industrial and Commercial Relations; Executive of the American Construction Council; Director of the New York Building Congress; Formerly Lecturer in the Graduate School of Business Administration, Harvard University. Cloth. Pp. xvi, 553 with appendix and index. Chicago and New York: A. W. Shaw Co., 1925.

BYWAYS TO HEALTH: DETOURING THE SCRAP-HEAP. By *Thomas D. Wood*, A.M., M.D., College Physician and Adviser in Health Education, Teachers College; Professor of Physical Education, Columbia University; Author of "Health and Education" and many Health Reports and Pamphlets; and *Theresa Dansdill*, A.M., Director of Health Education, North Carolina Tuberculosis Association; Author of "Health Training in Schools." Cloth. Pp. 198. New York and London: D. Appleton & Co., 1925.

THE PSYCHOLOGY OF VOCATIONAL ADJUSTMENT. By *Harry Dexter Kitson*, Professor of Education, Teachers College, Columbia University; Author of "How to Use Your Mind," and "A Manual for the Study of Advertising and Selling." Cloth. Pp. 273 with illustrations and index. Philadelphia, London, and Chicago: J. B. Lippincott Co., 1925.

SUBMUCOUS ENDOCAPSULAR TONSIL ENUCLEATIONS, WITH DISCUSSION OF THE EVOLUTION OF KNOWLEDGE OF THE TONSIL AS A DISEASE PRODUCING FACTOR AND VARIOUS METHODS OF ENUCLEATION. Excerpts from Clinics of *Charles Conrad Miller*, M.D. Cloth. Pp. 218. Chicago: The Oak Press, 1925.

EARS AND THE MAN. STUDIES IN SOCIAL WORK FOR THE DEAFENED. By *Annetta W. Peck, Estelle E. Samuelson, Ann Lehman;* with an Introduction by *Wendell C. Phillips*, M.D., President-Elect of the American Medical Association. Cloth. Pp. 217 with bibliography and index. Philadelphia: F. A. Davis Co., 1926.

BOOK REVIEW

THE HEALTH OF THE WORKERS. By *Sir T. Oliver*. Pp. 226. London: Faber & Gwyer, Ltd., 1925.

The pen of the well-known author of this small book has not lost its cunning. It contains, condensed into small space, a mass of information and yet remains readable and interesting. Written for the general public, for workers and employers, it yet presents a picture of occupational hygiene of great value for any practitioner busied in an industrial district. Only one widely read and deeply versed in his subject could so portray it. Information has been gathered not only from his own experience and travels in other countries, but from the work of others all over the world.

The position of industry in the community and its duties to it are sketched. No man's health should be harmed by his service to the state, which is his work. Then the extent to which industry falls short of its best is described in regard to sickness and accidents suffered, and to consequent inefficiency and unrest. The mining industry receives special attention. Diseases and poisonings incidental to occupations are told of, and means for their prevention is outlined. As is correct when writing for the public, advanced views are seldom presented; hence occasionally experts may differ with the author. But errors are few and the book may safely be recommended for perusal and reference.—*E. L. Collis.*

ANNOUNCEMENT OF MEETING OF THE AMERICAN ASSOCIATION OF INDUSTRIAL PHYSICIANS AND SURGEONS

The Eleventh Annual Meeting of the Association of Industrial Physicians and Surgeons will be held in Philadelphia, May 24 to 26, 1926.

In addition to the program given below, two amendments to the Constitution are proposed for adoption as follows:

1. That the fiscal year of the Association be changed from May to January 1.
2. That the Board of Directors be empowered to elect applicants to membership in place of such election by the Association.

An interesting and worth while program has been arranged by the committee and all members and interested friends are urged to attend.

Further details will be sent you later from the secretary's office.

F. L. Rector
Secretary-Treasurer

Program

Headquarters, Hotel Pennsylvania
Clinical sessions at the University of Pennsylvania

Monday, May 24, 1926

9:00 A.M.	Business meeting.
	Reports of committees.
	President's Address. *Dr. Wm. B. Fisk.*
10:00	The Problems of Industrial Medicine. *Dr. George M. Piersol,* American Telephone and Telegraph Company.
	Discussion.
11:00	Pension and Relief Benefit Associations.
	Leader to be announced later.
	General discussion.
12:00	Lunch. All members expected to attend.
1:30 P.M.	Chest and X-ray Clinic. *Drs. Pancoast and Landis.* At the Hospital of the University.
	Discussion.
8:00	Guests of the Industrial Medicine and Health Section of the College of Physicians, Philadelphia, at the College. Program arranged by the College.

Tuesday, May 25, 1926

By the Department of Surgery of the University of Pennsylvania

9:00 A.M. HEAD INJURIES. Surgical Clinic. *Dr. Charles Frazier*, Professor of Surgery.
10:00 FRACTURES. *Dr. Eliason* of the Surgical Staff.
11:00 THREE OPERATIVE CLINICS. *Dr. Frazier* and his Staff.
1:00 P.M. INDUSTRIAL DERMATOSES. Clinic. *Dr. Stokes*, Professor of Dermatology, and his Staff.
2:15 General lunch.
3:30 FRACTURES IN AND AROUND JOINTS. Dr. John J. Moorhead, New York Post Graduate School.
6:00 Dinner.
8:00 SURGICAL AND MEDICAL ROUND TABLES. (Speakers to be announced later.)
Surgical: Amputations; Trauma; Fractures.
Medical: What Are the Industrial Diseases? Relation of Industry to Disease.

Wednesday, May 26, 1926

By the Medical Department of the University of Pennsylvania

9:00 A.M. CIRCULATORY DISTURBANCES. Clinic. *Dr. Stengel*, Professor of Medicine.
Followed by a clinic by *Dr. Miller* of the Medical Staff.
12:00 Lunch.
1:15 P.M. SURGICAL CLINIC. *Dr. John B. Deaver*, Lankenau Hospital.
Orthopedic, eye, and medical clinics are also being arranged.

This is the program as outlined at this date. There will be some changes in the way of additional papers and discussions which will be announced later. It is planned to head up the round table discussions by the best men we can in the various lines suggested.

The Medical Department of the University of Pennsylvania has very kindly consented to be sponsor for the clinical work of the meeting and has invited us to use the Department in any way that we desire to make our meeting profitable.

The Industrial Section of the College of Physicians has invited us to be its guests for Monday evening.

It is the desire of your program committee to give you the best meeting in the history of the Association. In return we want every member of the Association to make every effort to attend.

Respectfully submitted,

Committee:
A. W. COLCORD
HARRY MOCK
LOYAL A. SHOUDY